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Molecular genetic aspects of neurodegenerative diseases

Školitel: prof. MUDr. Radoslav Matěj Ph.D.

Prohlášení:

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1. ÚVOD

Neurodegenerativní onemocnění jsou definovány jako stavy, při nichž dochází k postupnému zániku specifických skupin neuronů. Většinou se jedná o věkově vázaná onemocnění – klinicky se manifestují v dospělosti a jejich výskyt stoupá s rostoucím věkem. Jejich společným rysem je tvorba a ukládání určitého specifického – pro dané onemocnění typického – proteinu do mozkové tkáně v kombinaci s obecnými mechanismy apoptózy. Neurodegenerativní onemocnění můžeme vymezit jako specifické proteinopatie. Podle proteinů obsažených v depozitech jsou ještě rozdělovány na β-amyloidopatie, *tau*-proteinopatie, α-synukleinopatie, prionopatie a další.

Faktory, které jsou základem neurodegenerace, lze klasifikovat jako genetické, environmentální (vnější neurotoxiny, např. kovy, infekční poškození), biologické (agregace, abnormální skládání a akumulace proteinů), metabolické (oxidační stres), excitotoxické (vnitřní neurotoxiny, např. kovy), autoimunita a stárnutí. Každý z těchto faktorů může hrát klíčovou roli v etiologii neurodegenerativních onemocnění, ale s vlivem, který se mezi různými patologiemi liší (1).

Velmi významný – a pravděpodobně i primární – efekt je patologické ukládání abnormálních depozit jinak správně nasyntetizovaných a fungujících proteinů (např. β-amyloidu, tau proteinu, alfa-synukleinu, prionového proteinu) v mezibuněčném prostoru mozkové kůry, jež se stávají pro neurony toxické a spouštějí kaskádu dalších dějů (tvorbu volných kyslíkových radikálů, sterilní zánětlivou reakci, toxické působení excitačních aminokyselin s nadměrným vstupem vápníku do neuronů atd.). Výsledkem těchto dějů je zánik postiženého neuronu. Extracelulární proteinová depozita (plaky) jsou základem histopatologické diagnózy neurodegenerativních změn. Ještě dříve, než dojde k depozici amyloidu ve formě oblených agregátů charakteru plak, vznikají neurotoxické oligomery. Intracelulárně, v neuronech, dochází k abnormální fosforylaci proteinu τ s tvorbou tzv. neurofibrilárních klubek (smotků, tangles). Dochází k uvolňování zánětlivých cytokinů a řadě dalších reakcí. Výsledkem je pak podstatné snížení neuronální plasticity.

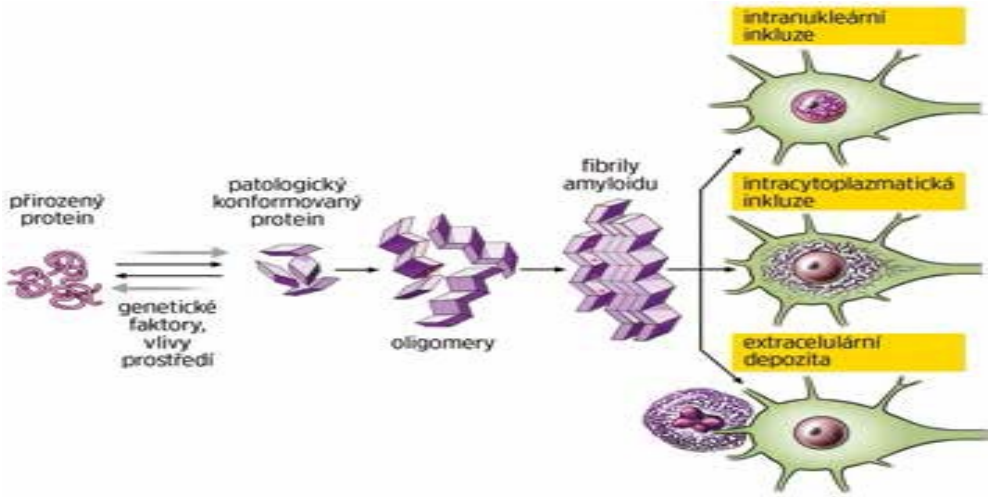
Hlavní patofyziologické mechanismy neurodegenerací

Neurodegenerativní onemocnění jsou způsobena kombinací většího počtu patogenetických vlivů, z nichž 4 hlavní jsou:

- apoptóza – souhrou vnitřních a vnějších spouštěcích mechanismů dojde k interakci pro- a antiapoptických faktorů, jež ve svém důsledku spustí kaskádovitou reakci s výsledným zánikem postižené buňky. Většina neurodegenerativních onemocnění není provázena zánětlivou reakcí.
- produkce volných kyslíkových radikálů – působení volných kyslíkových radikálů (ROS) je dáno kombinací zvýšené produkce nebo nedostatečnou inhibicí jejich působení v důsledku postižené enzymatické buněčné výbavy.
- abnormální patologické proteinové agregáty – jejich vznik, interakce a nemožnost fyziologické degradace je v úzkém vztahu s přítomností ROS a jejich vlivem na posttranslační modifikace proteinů. Porušené mohou být intracelulární enzymatické komplexy, např. ubikvitinového proteásového systému, které potencují vznik intracelulárních a extracelulárních depozit patologických proteinových agregátů (1).
- genetické pozadí - vliv různých genových polymorfizmů a postižení genomu patogenními mutacemi je jeden z hlavních patofyziologických mechanismů. Dědičně podmíněno může být až 15-20 % neurodegenerací a neustále přibývají nové varianty, které přímo či nepřímo souvisejí s jejich patofyziologií. Zvláštním případem nealelních interakcí je polygenní dědičnost. Každý z genů sám o sobě má malý účinek na fenotyp, a proto hovoříme o tzv. minor genech. Na tomto typu dědičnosti se podílí více genů, mezi kterými mohou být různě složité interakce – jen minimálně ovlivňovány zevním prostředím. V patogenezi neurodegenerativních onemocnění se ale oproti zjednodušenému polygennímu modelu předpokládá tzv. multifaktoriální dědičnost, která je založena na interakci genů velkého účinku (major geny) s polygenním systémem (tzv. genetické pozadí), která je navíc modulována různě intenzivním působením faktorů vnějšího prostředí. Uplatňuje se model vulnerabilita (spouštěcí faktor: čím je jedinec zranitelnější/predisponovanější (polymorbidita), tím menší podnět stačí k rozvoji onemocnění). Všechny neurodegenerativní poruchy mají významné genetické složky, přičemž genetická dědičnost pro Alzheimerovu chorobu (AN), Parkinsonovu chorobu (PN) a amyotrofickou laterální sklerózu (ALS) se odhaduje na 60–80 %, (2) ~ 40 % (3) a ~ 60 % (4). Mendelovské formy neurodegenerativních onemocnění se připisují vzácným mutacím v genech, jako je protein amyloidního prekurzoru (*APP*), v presenilinech (*PSEN1*, *PSEN2*), α-synuklein (*SNCA*), Parkin (*PARK2*), kináza I indukovaná PTEN (*PINK1*), protein spojený s mikrotubuly tau (*MAPT*), dardarin (*LRRK2*), protein B spojený s cytosolickou Cu/Zn

superoxiddismutázou (*SOD1*), alsin (*ALS2*), senataxin (*SETX*) a synaptobrevin/VAMP (membránový protein spojený s vezikulemi) (*VAPB*) pro ALS (5) a *C9orf72* (6). Většina případů onemocnění je však nemendelovské formy a vykazuje složitou etiologii zahrnující velké množství genetických variant se středními až jemnými účinky.

Obrázek č.1 Schéma obecného principu neurodegenerací (Rusina a Matěj 2019).



Klasifikace neurodegenerativních nemocí

V literárních zdrojích existuje řada klasifikací, které se od sebe liší. Jedno z možných dělení vychází z poznatků molekulárně neuropatologických studií. Zlatým standardem je třídění neurodegenerativních onemocnění z biochemického hlediska, do 7 základních skupin:

1. Alzheimerova nemoc

2. Frontotemporální lobární degenerace

3. Synukleinopatie

4. Onemocnění s opakováním tripletů

5. Prionová onemocnění

6. Onemocnění motorického neuron

7. Ostatní neurodegenerativní onemocnění

2. PŘEKRÝVÁNÍ NEURODEGENERACÍ

Alzheimerova choroba (AN), Parkinsonova choroba (PN), frontotemporální lobární degenerace (FTLD), amyotrofická laterální skleróza (ALS), Huntingtonova choroba (HN) a prionová onemocnění mají určitý stupeň klinického, patologického a molekulárního překrývání (7) (8) (9) (10). U těchto onemocnění byly pozorovány klinické a patologické podobnosti (7), což naznačuje, že různé typy neurodegenerací mohou být způsobeny překrývajícími se genetickými faktory (11) (12). Překrývající se klinické a neuropatologické vlastnosti neurodegenerativních forem demence vedou k chybné diagnóze. Pro přesnou diagnózu a pro odhad budoucího rizika je nezbytné genetické profilování (7) (10) i z důvodu, že tato onemocnění mají složité genetické pozadí.

Různé neurodegenerace se mohou vyskytovat současně a pro tyto stavy se v současné době používají tři termíny: souběžná neurodegenerativní onemocnění, souběžná neurodegenerativní patologie a smíšená neuropatologie, respektive smíšená demence (1) .

Pojem souběžná neurodegenerativní onemocnění (concomitant neurodegenerative diseases) je požíván tam, kde vedle jedné potvrzené vyvinuté neurodegenerace lze nalézt i projevy další plně rozvinuté neurodegenerativní entity. Jde o skutečnou kombinaci dvou odlišných onemocnění (např. Alzheimerovy choroby a multisystémové atrofie) (1).

Příklady souběžné neurodegenerativní překryvné diagnózy

- Alzheimerova nemoc a multisystémová atrofie
- Alzheimerova nemoc a Pickova nemoc
- Alzheimerova nemoc a progresivní supranukleární obrna
- Alzheimerova nemoc a demence s Lewyho tělísky

Souběžná neurodegenerativní patologie (concomitant neurodegenerative pathology) charakterizuje stav, kdy v určitých oblastech mozku nacházíme plně rozvinutou primární neurodegenerativní entitu zároveň s přítomností depozit proteinu specifického pro jinou neurodegeneraci, přičemž jsou neuropatologická kritéria splněna jen pro primární neurodegeneraci (např. AN a depozita proteinu TDP-43) (13).

Příklady souběžné neurodegenerativní překryvné patologie

- FTLD-TDP s depozity alfa-synukleinu
- Alzheimerova nemoc s depozity TDP-43 v hipokampu a amygdale
- Alzheimerova nemoc a Lewyho tělíska a neurity v amygdale

Smíšená demence (mixed dementia) je požívána pro kombinaci neurodegenerativního onemocnění a vaskulární patologie (14). Pravděpodobně nejčastější kombinací neurodegenerativních onemocnění sensu lato je kombinace vaskulární patologie a jiného, primárního neurodegenerativního onemocnění, zejména AN či demence s Lewyho tělísky (DLB). Pravděpodobnost výskytu cerebrovaskulárních změn navíc narůstá s věkem. Vaskulární patologie z neuropatologického hlediska zahrnuje celou řadu lézí, od makroskopicky zcela jasně identifikovatelných lézí s odpovídajícím klinickým korelátem až po mikroskopické léze, jejichž význam je nejistý (15).

Příklady smíšené překryvné demence

- Alzheimerova nemoc a vaskulární demence
- Demence s Lewyho tělísky a vaskulární demence

3. PRIONOVÁ ONEMOCNĚNÍ

Prionová onemocnění jsou přenosné, progresivní a fatální neurodegenerativní poruchy spojené s agregací nesprávně složeného prionového proteinu (PrP). Prionový protein (PrP^C) má fyziologickou funkci jako sialo-glykoprotein s buněčnou membránou ukotvenou v glykolipidech lokalizovaný v presynaptických membránách s neuroprotektivní a promyelinizační rolí. Prionová onemocnění jsou spojena s patologickou samoreplikační konformací PrP, jejímž základem je změna terciární struktury PrP posttranslačním procesem do převládající struktury β-listu. Tento proces vede k tvorbě výrazně hydrofobní varianty PrP s jasnou tendencí k agregaci, následné oligomerizaci a tvorbě amyloidových plaků (16). Vzniklé patologické agregáty PrP^{Sc} jsou extrémně odolné vůči fyzikálním nebo chemickým účinkům a na rozdíl od většiny proteinů jsou tyto molekuly nedenaturovatelné dosažením bodu varu.

3.1. LIDSKÁ PRIONOVÁ ONEMOCNĚNÍ

3.1.1. Lidské přenosné spongiformní encefalopatie (TSE)

Lidská prionová onemocnění jsou definována jako přenosná a rychle progresivní degenerativní onemocnění centrálního nervového systému způsobená akumulací patologicky přizpůsobeného PrP s různě dlouhou inkubační dobou a většinou rychlým průběhem s infaustní prognózou způsobené priony (proteinous infectious particles). Jedná se o bílkoviny, které se normálně vyskytují na povrchu řady buněčných struktur, jsou kódovány na *PRNP* genu 20. chromozomu (17). Mezi lidské spongiformní encefalopatie patří Creutzfeldtova – Jakobova choroba (CJD), Gerstmannův – Sträusslerův – Scheinkerův syndrom (GSS), kuru a fatální familiární nespavost (FFI) (18). Nejčastějším zástupcem prionových onemocnění v populaci je Creutzfeldtova-Jakobova nemoc, která se vyskytuje ve sporadické, dědičné (familiární) a infekční (náhodně přenesené, iatrogenní) formě. Nová varianta Creutzfeldtovy-Jakobovy nemoci je pak dávána do souvislosti s alimentární expozicí (konzumace hovězího masa z dobytka nakaženého bovinní spongiformní encefalopatií, BSE), s krevní transfuzí z nemocných dárců.

3.1.2. Creutzfeldtova-Jakobova choroba

Creutzfeldtova-Jakobova choroba je nejčastějším zástupcem lidských prionových onemocnění. Čtyři typy - nejčastější sporadická (sCJD), familiární (fCJD), iatrogenní (iCJD) a nová varianta (vCJD) - se rozlišují podle různých etiologií (19). Prvními příznaky mohou být drobné výpadky paměti. V počátečních stádiích jsou také časté změny nálady, ztráta zájmu o okolí a neochota účastnit se společenského života. Následuje pokles pracovní výkonnosti s tím, jak se pro nemocného stávají stále složitějšími i úkoly, které dosud hravě zvládal. V této fázi se může choroba jevit jako deprese. Během několika týdnů nemocný ztrácí stabilitu při chůzi, trpí

poruchami vidění, mohou se dostavit i halucinace. Choroba poznamenává i řeč. Člověk mluví pomalu, nezřetelně vyslovuje a při konverzaci s obtížemi hledá vhodná slova. Nakonec se už nedokáže ani pohybovat ani mluvit. Proces se sice pomalu, ale nezadržitelně šíří mozkovou tkání, není doprovázen imunitní odezvou, ale mikroskopický obraz poškozené tkáně je typický pro spongiformní encefalopatie. Definitivní diagnóza CJD je založena na neurohistologickém vyšetření doplněném imunohistochemickými metodami metodou western blot a molekulárně genetickým vyšetřením. Při pozitivitě neurohistologického vyšetření se nachází trojice změn: spongiformní dystrofie, úbytek neuronů a astroglióza. Imunohistochemické vyšetření spočívá v zjišťování komplexů různých typů protilátek barvením. Metoda Western blot se provádí již v průběhu onemocnění, jedná se o nález nespecifického proteinu 14-3-3 v mozkomíšním moku. V závislosti na typu proteinázových K-rezistentních fragmentů prionového proteinu (PrP) vyšetřovaných metodou Western blot rozlišujeme podle Parchi a spol. mezi PrP typu 1 a 2 (20).

3.1.3. Sporadická CJD

Sporadická varianta (sCJD) je vzácné progredující neurodegenerativní onemocnění s infaustní prognózou, které se projevuje rychle postupující demencí a řadou dalších neurologických příznaků. Začíná náhodnou konverzí fyziologického PrP^{C} na patologicky potvrzený PrP^{Sc} a tvoří asi 85 % případů CJD. Celosvětový výskyt je uváděn jako 1-2 případy na milion obyvatel na rok (21). Onemocnění trvá méně než dva roky (zpravidla průběh nepřekračuje rok), delší trvání je vylučujícím klinickým kritériem pro sCJD (22).

3.1.4. Familiární CJD (genetická)

Familiární / genetická varianta (fCJD) je podmíněna přítomností mutace v genu pro prionový protein *PRNP*, která se týká 10–15 % případů CJD (23). Existuje více než 50 známých mutací. V České republice se setkáváme u většiny familiárních forem s mutací E200K, což je také nejběžnější mutace v Evropě obecně, v evropské populaci následované mutacemi V210I a D178N. Mutace D178N je lokálně relativně běžná v západní Evropě (Nizozemsko, Francie, Velká Británie), ale také ve Finsku a Maďarsku (23).

3.1.5. Získaná CJD (iatrogenní, náhodně přenášená)

Iatrogenní varianta (iCJD) vzniká během lékařských nebo chirurgických zákroků, při nichž byly přeneseny patologicky konformované priony. iCJD je extrémně vzácná a vyskytuje se u méně než 1% všech případů CJD. Onemocnění bylo zjištěno po transplantacích rohovky, po použití nedostatečně sterilizovaných intracerebrálních elektrod, transplantacích dury mater (DM), dále užitím růstového hormonu a gonadotrofinu získaného z kadaverozních hypofýz.

Inkubační doba iCJD u příjemců DM) z celého světa se pohybuje od 1,3 do 30 roků (Ø 12 let). Je zajímavé, že existují 3 případy pravděpodobného přenosu vCJD prostřednictvím krevních transfuzí od dárce trpícího vCJD - z tohoto důvodu platí zákaz dárců, kteří žili ve Velké Británii během epidemie BSE (24).

3.1.6. Variantní CJD

Toto onemocnění (vCJD) je vysoce pravděpodobně spojeno s konzumací hovězího dobytka postiženého bovinní spongiformní encefalopatií (BSE).

V klinické prezentaci dominují na začátku psychiatrické a behaviorální příznaky s bolestivou parestézií nebo dysestézií, zatímco ataxie a demence se vyvinou později (25). V České republice toto onemocnění doposud nebylo prokázáno.

3.1.7. Gerstmannův – Sträusslerův – Scheinkerův syndrom

Gerstmannův – Sträusslerův – Scheinkerův syndrom (GSS) je definován jako pomalu progredující dědičné autosomálně dominantní neurodegenerativní onemocnění nebo encefalo(myelo)patie s multicentrickými plaky PrP lokalizovanými v mozkové a mozečkové kůře a bazálních gangliích. (26).

GSS byl navíc první lidský TSE se známou mutací *PRNP*, dnes zahrnující bodové mutace v kodonech 102, 105, 117, 131, 145, 187, 198, 202, 212, 217, 232 nebo inzerci opakování oktapeptidu (OPRI) s počtem 1–9 z 24 násobků párů bází. V České republice se setkáváme zatím výhradně s mutací P102L (27).

3.1.8. Fatální familiární insomnie

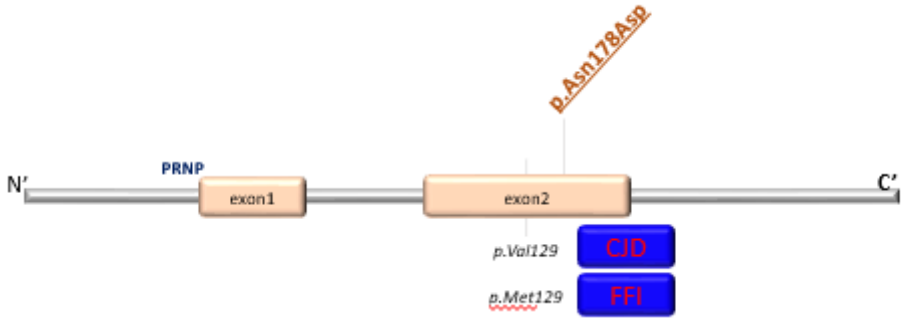
Fatální familiární insomnie (FFI) je autozomálně dominantní dědičné onemocnění způsobené mutací D178N v genu *PRNP* spojenou s přítomností polymorfizmu MM na kodonu 129. FFI je charakterizována nespavostí rezistentní na léky, fragmentací spánku, poruchou autonomního nervového systému, motorickými poruchami a progresivní kognitivní porucha (28). Hlavními postiženými oblastmi jsou mediodorsální a přední ventrální thalamická jádra, následovaná pulvinarem a olivami. Hlavními neuropatologickými nálezy jsou rozsáhlá ztráta neuronů a astrocytová glióza, zatímco spongiformní transformace chybí. FFI se v České republice zatím nevyskytl (29).

3.1.9. Vnímavé polymorfismy

Významnou roli v patofyziologii u některých neurodegenerativních onemocnění mají vedle vlastní patogenní mutace i polymorfizmy v příslušném genu. Genetický polymorfizmus je

definován existencí dvou nebo více alel (variant genů) v jednom lokusu, převyšující svým výskytem 1% výskyt v populaci. Polymorfizmus samotný onemocnění nezpůsobí, ale může ovlivnit věk, kdy se objeví první příznaky, může souviset s vyšší vnímavostí jedince k onemocnění, modifikovat individuální rozvoj onemocnění nebo ovlivnit jeho průběh – pozoruhodným příkladem je mutace v kodonu 178 (N178D) genu prionového proteinu, která se projeví jednou jako hereditární Creutzfeldtova-Jakobova nemoc pokud je na mutované alele v kodonu 129 metionin a podruhé jako fatální familiární insomnie (pokud je na mutované alele v kodonu 129 valin (obr.č.2).

Obrázek č.2. Mutace v kodonu 178 (N178D) genu prionového proteinu



3.2. NEUROPATOLOGIE PRIONOVÝCH CHOROB

Definitivní diagnóza lidských prionvých chorob je založena na neurohistologickém vyšetření mozkové tkáně včetně imunohistochemických metod a Western blotem. Součástí diagnózy je i molekulárně genetické vyšetření, kdy se sekvenuje gen *PRNP*. Klasický neurohistologický obraz tvoří trojce změn – spongiformní dystrofie, numerická atrofie neuronů a sekundární reaktivní izomorfní astroglióza. Spongiformní dystrofii charakterizují vakuoly v neuropilu. Patogenní priony lze v mozkové tkáni prokázat různými typy protilátek, přičemž uvedené změny postihují zejména kortex, subkortikální šedou hmotu a stratum moleculare mozečku (1).

3.3. GENETIKA PRIONOVÝCH CHOROB

Onemocnění je způsobeno mutací v *PRNP* genu, bylo popsáno kolem dvaceti různých mutací vedoucích zpravidla k záměně jedné aminokyseliny za jinou v řetězci PrP. Přítomnost mutace *PRNP* je nezbytná pro stanovení diagnózy genetického prionového onemocnění u

symptomatického jedince. Bylo nalezeno několik bodových mutací *PRNP* ve spojení s fenotypem CJD - Asp178Asn, Val180Ile, Thr183Ala, Glu196Lys, Glu200Lys, Val203Ile, Arg208His, Val210Ile, Glu211Gln, Met232Arg. Fenotyp GSS mohou zahrnovat mutace - Pro102Leu, Pro105Leu, Ala117Val, Gly131Val, Tyr145Stop, Gln160Stop, Phe198Ser, Asp202Asn, Gln212Pro a Gln217Arg. Fenotyp FFI může způsobit pouze jeden haplotyp Asp178Asn + normální varianta M129. Octapeptidové opakované inzerce jsou spojeny s různým klinickým a patologickým fenotypem. Všechny tyto inzerce leží v nestabilní oblasti *PRNP*, která je bohatá na prolin, glycin a glutamin. Normální alely *PRNP* mají jeden nonapeptid následovaný čtyřmi sekvencemi oktapeptidových opakování, z nichž každá obsahuje následující aminokyseliny: Pro- (His / Gln) -Gly-Gly-Gly - (- / Trp) -Gly-Gln (30).

Kodon 219 - varianta Glu219Lys byla hlášena zhruba u 6 % japonské populace. Ve studiích na transgenních myší bylo zjištěno, že PrP-219K nebyl přeměněn na PrP^{Sc} a také inhiboval konverzi společně exprimovaného nemutovaného typu PrP. Pravděpodobně se jedná o alelu s protektivní funkcí (31).

Kodon 127 - nedávno byl u selektivní populace na Nové Guineji identifikován nový polymorfizmus Gly127Val. Bylo navrhnuto, že tento polymorfizmus kromě kodonu 129 snižuje relativní riziko rozvoje kuru u exponovaných jedinců (31).

Kodon 129 – hraje významnou roli a je kódován methioninem (M) nebo valinem (V). Největší vnímavost vůči prionovým onemocněním mají homozygoti MM nebo VV. Menší vnímavost mají heterozygoti MV. Významná je role polymorfizmu kodonu 129, pokud je současně přítomna mutace na kodonu 178. Mutace D178 způsobuje fCJD, pokud se současně na pozici 129 mutované alely D178N vyskytuje aminokyselina valin. Metionin na 129 pozici společně s mutací D178N je příčinou FFI.

Penetrance – *PRNP* mutace Glu200Lys a Val210Ile jsou obvykle spojovány s proměnlivou, ale obecně věkově závislou penetrancí, takže čím je jedinec starší, tím větší je pravděpodobnost manifestace nemoci.

3.4. KOMBINACE NEURODEGENERACÍ U PRIONOVÝCH ONEMOCNĚNÍ

Přítomnost prionového onemocnění nevylučuje možnost další souběžné neurodegenerace, přičemž doprovodná neurodegenerace nemusí být správně rozpoznána. Relativně častější je u genetických forem CJD, zejména u specifické formy s mutací E200K. Nejčastější souběžnou neuropatologií je patologie tau proteinu, která může, vč. přítomnosti difúzních či neuritických plak, splnit diagnostická kritéria pro diagnózu PART (primární na věk vázaná tauopatie) nebo

AN. Jako u dalších neurodegenerativních onemocnění nelze ani u prionových onemocnění prakticky vyloučit možnost kombinace dvou neurodegenerací či souběžné neuropatologie. Popisovány jsou kombinace Creutzfeldtovy-Jakobovy nemoci s AN, demence s Lewyho tělísky nebo dokonce multisystémové atrofie (MSA) (32) (33).

4. AMYOTROFICKÁ LATERÁLNÍ SKLERÓZA

Amyotrofická laterální skleróza (ALS), známá též jako Lou Gehrigova choroba, Charcotova nemoc či onemocnění motorického neuronu) je progresivní, fatální, neurodegenerativní onemocnění motorických neuronů mozku a míchy, způsobující degeneraci a ztrátu mozkových (horních) a spinálních (dolních) motorických neuronů. Většina pacientů s ALS umírá během 2 až 5 let od prvních příznaků, nejčastěji na respirační selhání. Incidence ALS se v Evropě uvádí kolem 1-2/100 000 obyvatel za rok (34). Obvykle jsou postiženy osoby mezi 60 a 70 lety, vzácně před 40. ALS se vyskytuje nejčastěji v sporadické formě (sALS), zatímco familiární forma (fALS) se vyskytuje v 10-15 % (35). U pacientů s ALS a demencí pravidelně nacházíme TDP-43 – pozitivní inkluze, a proto bylo zavedeno klinické a neuropatologické označení FTLD-MND-TDP (frontotemporal lobar degeneration with motor neuron disease and TDP-43 positive inclusion). Toto onemocnění patří do širokého spektra fronto-temporálních lobárních degenerací.

Diagnostická kritéria dle Stronga et al. z roku 2017 kladou z kognitivního hlediska důraz na postižení sociální kognice (kontrola emocí, chování přiměřené kontexu a situaci, sociální kontakty a vztahy), řeči a paměti i na přotomnost neuropsychiatrických projevů (36). Tyto manifestace jsou důsledkem frontotemporální dysfunkce asociované s ALS. Proto se v klinické praxi spíše používá označení frontotemporální spektrum postižení u ALS (ALS-FTSD) (amyotrophic lateral sclerosis – frontotemporalspectrum disorder).

Další inkluze, které byly identifikovány v motorických neuronech u ALS, jsou ubikvitin pozitivní inkluze. Ty byly pozorovány u degenerujících motorických neuronů u pacientů s ALS bez inkluzí proteinu TDP-43. Mutace spojené s ALS v genu *UBQLN2* (gen kódující ubikvitin) souvisejí s dysfunkcí autofagie, neurozánětu a tvorbou stresových granulí (SG). Další, mnohem vzácnější formou ALS, je agregace FUS proteinu v motorických neuronech (37).

4.1. NEUROPATOLOGIE AMYOTROFICKÉ LATERÁLNÍ SKLERÓZY

V regresivně změněných motorických neuronech stejně jako v neuronových strukturách frontálního a temporálního kortexu bývají přítomny různé typy inkluzí. Některé jsou imunohistochemicky pozitivní s použitím protilátek proti ubikvitinu a proteinu p62. Pozitivitu vykazují i inkluze v cytoplazmě při použití protilátky proti TDP-43, zejména jeho hyperfosforylovaná forma. U FTLD-TDP-MND jsou inkluze zastoupeny nejvíce ve frontální a temporální kůře. U ALS se zachovalou kognicí jsou depozita v cytoplazmě motorických

neuronů. U komorbidit jsou přítomné inkluze specifické pro AN (depozita beta-amyloidu a neurofibrilátní klubka) nebo rozsáhlé vaskulární postižení (glióza, numerická atrofie neuronů) (38) (39).

4.2. GENETIKA AMYOTROFICKÉ LATERÁLNÍ SKLERÓZY

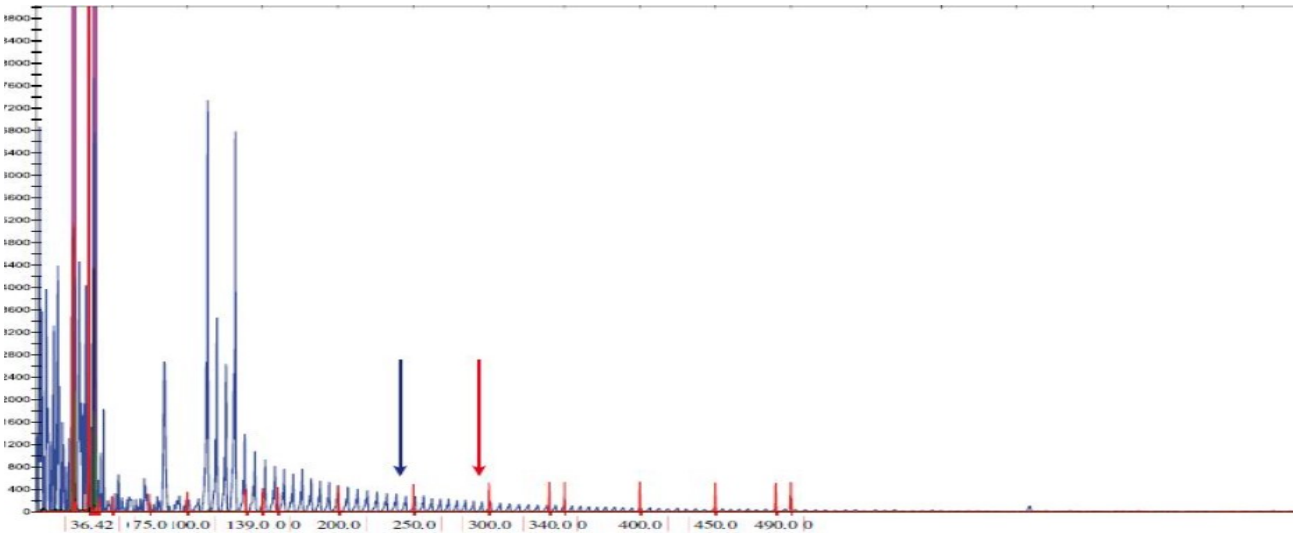
Genetika ALS je velmi podrobně popsána v experimentální části.

I přesto, že propojení ALS a frontotemporální demence (FTD) pochází z roku 1932, patologický podklad tohoto přesahu se podařilo poodkrýt až v posledních letech.

Superoxiddismutáza Cu / Zn nebo *SOD1* byl prvním genem spojeným s ALS, přičemž nedávný pokrok v genetické diagnostice vedl k objevení dalších genetických markerů pomocí GWAS (genome-wide association study). Geny související s onemocněním znázorňuje tabulka č.1. Nejčastější jsou *TARDBP, SQSTM1, ROS, VCP, FUS, TBK1, CHCHD10, TBK1, TUBA4A, CCNF, MATR3, NEK1, C21orf2, ANXA11, TIA1* a zejména *C9orf72* (40).

Genetické studie u pacientů s familiárním současným výskytem frontotemporální lobární demence a amyotrofické laterální sklerózy (FTLD-ALS) odhalily expandovanou repetici v otevřeném čtecím rámci 72 chromozomu 9. Více jak 50 % pacientů s familiární formou FTLD-ALS a 15-20 % pacientů se zdánlivě sporadickou formou FTLD-ALS jsou nositeli expandované repetice genu *C9orf72* (41). Překvapujícím faktem o genetické mutaci na genu *C9orf72* je klinická variabilita symptomů a prognózy. V souvislosti s touto mutací byl již pozorován výskyt parkinsonismu, Alzheimerovy choroby, psychiatrických nemocí a nemoci podobné Huntingtonově choreе. Příčina této fenotypové variability je neznámá (42). Přestože je s mutací na genu *C9orf72* často spojena rychlá progrese FTLD-ALS, byly již zaznamenány i případy, kdy pacienti měli pomalou progresi a projevovaly se u nich příznaky pouze FTD. Protein kódovaný *C9orf72* souvisí hlavně s autofagií, andozomálním transportem a imunitní funkcí. Podle statistik obsahovalo rozšířené alely *C9orf72* asi 40-50 % fALS a 10 % sALS. Patogenní alely *C9orf72* mohou mít stovky nebo dokonce tisíce repetice hexanukleotidu GGGGCC. Velké množství klinických studií ukázalo, že do intronu umístěného mezi dvěma nepřekládanými volitelnými exony 1a a 1b genu *C9orf72* (43) (44) (45) je vloženo přibližně 700-1600 GGGCC hexanukleotidových opakování. Penetrance choroby ALS související s *C9orf72* je považována za téměř 100 % ve věku 80 let. U ALS – FTLD lze také pozorovat metabolické změny v proteinu tau, zejména patologickou fosforylaci Thr175 (pThr175tau) (46).

Obrázek č.3 Hexanukleotidová expanze *C9orf72*.



Určení počtu kopií hexanukleotidové expanze C9orfF72. Referenční hodnoty: normální alela < 20 GGGGCC repetic, permutovaná alela 20–100 GGGGCC repetic, úplná penetrance > 100 GGGGCC repetic. Modrá šipka ukazuje místo 20 GGGGCC repetic a červená znázorňuje místo 30 a více repetic.

Jedním z důsledných patologických nálezů v případech ALS a FTD je přítomnost silně ubikvitinovaných neuronálních cytoplazmatických inkluzí, u nichž bylo v roce 2006 zjištěno, že obsahují TDP-43 kódovaný genem *TARDBP* (47). Mutace v genech kódujících proteiny TDP-43 (*TARDBP*) a *FUS* jsou obvykle spojeny s ALS. Mutace *TARDBP* byly původně identifikovány (48) jako přímý důsledek identifikace proteinových druhů odvozených od TDP-43 jako hlavní složky agregátů nalezených v horních a dolních motorických neuronech pacientů s ALS bez mutací *SOD1* a ve FTLD-UPS (49) (50). Zatímco 5 % rodinných pacientů s ALS má mutaci *TARDBP*, mutace se zřídka vyskytují u FTLD a FTD-MND (51).

S ALS byl spojen další protein pro zpracování RNA, mediátor exportu RNA GLE1 (Nucleoporin GLE1) (52). Funkční vztah mezi GLE1 a TDP-43 však zůstává nejednoznačný. Jednou z hypotéz je, že GLE1 se podílí na jaderném exportu cílů RNA TDP-43 a FUS (52). Nosiče opakování expanze *C9orf72* také vyvíjejí proteinopatii TDP-43 v postižených oblastech mozku a motorických neuronech (53). Nedávno bylo prokázáno, že charakteristické cytoplazmatické inkluze DPR (dipeptide repeats) nosičů opakovaných expanzí *C9orf72* inhibují jaderný import TDP-43 (54).

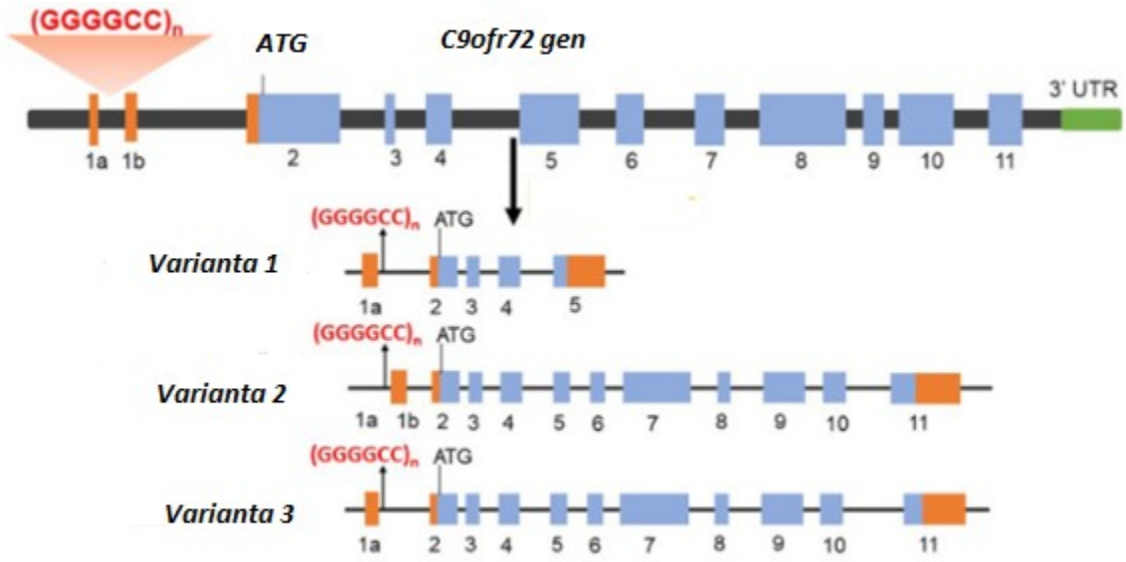
Kromě genů pro zpracování RNA mohou mutace *VCP, UBQLN2, SQSTM1, OPTN, CCNF, TBK1 a ANXA11* narušit degradaci proteinů prostřednictvím systému ubikvitin-proteazom-

vezikulárního transportu a autofagie, které pravděpodobně regulují odstranění TDP-43 z cytoplazmy (55) (56). Je pozoruhodné, že mnoho genů ALS bylo spojeno s odlišnými klinickými fenotypy, jako jsou proteinopatie svalů a kostí (*VCP, HNRNPA1, HNRNPA2B1, MATR3, TIA1*), (spino) cerebelární ataxie (*ATXN2, CHCHD10*), mitochondriální myopatie (*CHCHD10*) a autoimunitní poruchy (*ANXA11*).

Tabulka č.1 Patofyziologie ALS a zodpovědné geny

Mechanismus	Mutované geny
Dynamika cytoskeletu	PFN1, TUBA4A, DCTN1 a KIF5A
Zpracování RNA	C9orf72 , TDP-43, FUS a MATR3
Homeostáza bílkovin	UBQLN2, VCP, OPTN a VAPB

Obrázek č.4 Stuktura *C9orf72* genu (Swinnen B., 2018)



Struktura genu C9orf72 a navrhované mechanismy toxicity související s C9orf72 při řízení patogeneze ALS / FTD. C9orf72má 11 exonů a je transkribován do tří různých mRNA transkriptů: varianta 1 vede k translaci krátké proteinové izoformy, zatímco varianty 2 a 3 generují dlouhou proteinovou izoformu. Expanze hexanukleotidové repetice je přítomna v oblasti intronu 1 a je zachována v transkriptu pre-mRNA ve variantách 1 a 3, zatímco je přítomna v promotorové oblasti ve variantě 2.

5. ALZHEIMEROVA CHOROBA

Alzheimerova choroba (AN) je komplexní porucha centrálního nervového systému (CNS), u kterého dochází extracelulárně k akumulaci beta-amyloidu a intracelulárně k ukládání depozit hyperfosforylované formy tau proteinu do neurofibrilárních klubek (tangles). τ-protein se v defosforylovaném nebo částečně fosforylovaném stavu váže na mikrotubuly, které zpevňuje. Za patologických okolností jsou z τ-proteinu odštěpeny krajní aminokyseliny a je hyperfosforylován. Dalším klíčovým obrazem je numerická atrofie neuronů, která vede k atrofii mozkové tkáně doprovázené sekundární reaktivní astrogliózou.

5.1. NEUROPATOLOGIE ALZHEIMEROVY CHOROBY

Součástí buněčné membrány je bílkovina – amyloidový prekurzorový protein (*APP*), která plní různé fyziologické funkce. U nemocných s AN dochází ke štěpení *APP* pomocí beta- a gama-sekretáz, místo alfa-sekretázou. Tímto štěpením vzniká obtížně degradovatelný beta-amyloid, který se ukládá do mozkové tkáně ve formě amyloidových plak. Distribuce beta-amyloidu v mozkové tkáni příliš nekoreluje s klinickými příznaky (57). Navíc dochází k depozici beta-amyloidu do stěny cév a perivaskulárního prostoru pod obrazem cerebrální amyloidové angiopatie (CAA). Později dochází k tvorbě nerozpustných neurofibrilárních klubek z hyperfosforylovaného tau proteinu. Tím dojde k narušení axonálního transportu a ke kolapsu mikrotubulů (58). Definitivní diagnóza AN je založena na semikvantitativním hodnocením přítomnosti neuropatologických markerů v různých oblastech mozku. ABC kritéria dle NIA (National Institute of Aging) hodnotí množství amyloidu dle Thala, distribuci depozit tau proteinu podle Braakových a semikvantitativní distribuci neuritických plak dle CERAD (57).

5.1.1. Alzheimerova nemoc a tauopatie

Komorbidity AN s tauopatií jsou poměrně vzácné, relativně častější jsou kombinace AN s progresivní supranukleární obrnou (PSP) či kortikobazální degenerací (CBD). Výjimečná je kombinace AN a Pickovy nemoci nebo gliové globulární tauopatie (59). S rostoucím věkem roste i podíl nálezů ne zcela nespecifické a jednoznačně zařaditelné neuropatologie s depozity proteinu tau, která se může svými rysy blížit PSP, CBD, demence s argyrofilními zrny (AGD) i AN. Klinický nález u těchto pacientů je různý, často nespecifický s postižením poruchy paměti, chování, nálady, můžeme pozorovat parkinsonismus a jiné poruchy motoriky. Tyto patologické stavy přibývají s věkem a byly popsány jako – primární na věk vázaná tauopatie (PART) a věkově vázaná astrogliopatie s depozity tau proteinu (ARTAG) (60). Přítomnost PART a ARTAG byla prokázána i u prionových onemocnění, přičemž výskyt těchto onemocnění, jinak typické pro pokročilý věk, je i u mladších jedinců (61).

5.1.2. Alzheimerova nemoc a amyloidová angiopatie

U AN jsou typická kortikální a subkortikální depozita beta-amyloidu (plaky). Beta amyloid se rovněž ukládá do stěn arteriol s rozvojem cerebrální amyloidové angiopatie. Typickým projevem jsou mikrohemoragie v subkortikálních oblastech. CAA se může také vyskytovat i u kognitivně zdravých osob (62).

5.1.3. Alzheimerova nemoc a synukleinopatie

Jedná se o častou a komplikovanou kombinace alzheimerovské patologie s inkluzemi alfa synukleinu. V poslední době se objevují studie o propojení patologie tau proteinu a alfa-synukleinu. Patologicky konformovaný alfa-synuklein může indukovat změnu konformace tau proteinu (tzv. templated misfolding, prinoidní nebo prion-like mechanismus) (63).

5.1.4. Alzheimerova nemoc a depozita TDP-43

Souběžná neurodegenerativní patologie TDP-43 inkluzí u prokázané AN je relativně častá. Až u 50% případů AN lze TDP-43 inkluze nalézt prakticky vždy v amygdale, v hipokampálních strukturách, zejména ve spojitosti s hipokampální sklerózou (zde se však nejedná o hipokampální sklerózu ischemické etiologie asociovanou s epilepsií, ale o hipokampální sklerózu způsobenou degenerací pyramidových neuronů cornu ammonis v rámci primární neurodegenerace, např. AN), v subkortikálních jádrech, ve kmeni i v korových oblastech. Časně se TDP-43 depozita objevují v amygdale a s další progresí AN postupují přes hipokampální struktury dále do podkorových a korových oblastí (64). Inkluze hyperfosforylovaného TDP-43 jsou jedním z projevů stárnutí. Diskutován je jejich podíl na rozvoji věkem podmíněné hipokampální sklerózy, která se vyznačuje úbytkem neuronů a gliózou v cornu Ammonis. Tyto změny byly objektivizovány a byla ustanovena nová jednotka – věkově vázaná limbická TDP-43 proteinopatie (LATE) (65). Patologie TDP-43 může spolu s patologickými změnami v rámci AN synergisticky potencovat kognitivní deterioraci, podobně jako je tomu u vaskulární patologie či AGD. Na druhou stranu, přítomnost TDP-43 v bazálních gangliích a substantia nigra nevedl k významnějšímu výskytu parkinsonských příznaků či FTD (66).

5.2. GENETIKA ALZHEIMEROVY CHOROBY

Identifikace a funkční charakterizace autozomálně dominantních mutací v genu amyloidního prekurzorového proteinu (*APP*) a genech presenilinu 1 a 2 (*PSEN1* a *PSEN2*) podstatně přispěly k našemu porozumění biologickým mechanismům vedoucím k neurodegeneraci CNS u AN. Velká část genetické etiologie však zůstává nevyřešena, zejména u běžnějších sporadických forem AN. I když bylo vynaloženo značné úsilí na identifikaci genetických rizikových faktorů, které jsou základem sporadické AN, s použitím pečlivě navržených studií

genetické asociace u velkých skupin kontrolujících pacienty, jediným pevně stanoveným rizikovým faktorem zůstává alela ε4 genu apolipoproteinu E (*APOE)* (67).

5.2.1. Gen APP

APP se skládá z 18 exonů, s částí exonů 16 a 17 kódujících Ap peptid. Alternativní sestřih produkuje tři hlavní izoformy z nichž APP695 je převážně exprimována v mozku, zejména v neuronech (68). Všechny patogenní missense mutace *APP* se nacházejí v sekvenci Ap nebo v její blízkosti a v blízkosti míst štěpení proteázou, přičemž uplatňují svůj patogenní účinek ovlivněním proteolytického zpracování. Studie zkoumající selektivitu štěpení β-sekretázou *APP* však ukázala, že blokování β'-místa zavedením umělé dvojité mutace Y681K / E682K posunulo β-sekretázové štěpení (69).

5.2.2. Geny PSEN1, PSEN2

Většina mutací v genu *PSEN1* způsobuje typickou AN, klinicky a patologicky nerozeznatelnou od sporadické AN, s výjimkou raného věku a rychlejší a výraznější progrese onemocnění. Mutace v presenilinech přímo ovlivňují proces štěpení *APP* a vedou ke zvýšené produkci beta -amyloidu-42. Mutace v genu *PSEN1* jsou plně penetrantní a obvykle způsobují rychlou progresi onemocnění v mladším věku (v průměru kolem v 45 let) a jsou spojeny s AN s počátečním věkem> 65 let (70). Mutace v genu *PSEN2* jsou spojeny s pozdějším věkem nástupu onemocnění (45 až 88 let), penetrance je neúplná, kdy u některých osob, ačkoli genetickou vlohu k onemocnění nesou, se onemocnění nemusí vůbec klinicky manifestovat (71).

5.2.3. Gen APOE

Apolipoproteiny slouží jako informační molekuly, které zajišťují vazbu lipoproteinu na specifická místa; v mozku mají vliv na neuronální reparaci, růst dendritů, synaptickou plasticitu (především apolipoproteiny E) a byly popsány i jeho protizánětlivé účinky (72). Gen pro apolipoprotein E (*APOE*) je značně polymorfní gen a vyskytuje se ve 3 kodominantních alelách:

- alela E2 (s četností asi 7 %) způsobuje pomalejší odbourávání VLDL (vysokodenzní lipoprotein) a chylomiker a má nižší afinitu pro LDL (nízkodenzitní lipoprotein) receptory s nízkou absorpcí cholesterolu; je často spojena s hyperlipoproteinémií typu III, podílí se také na rozvoji Parkinsonovy nemoci
- alela E3 (s četností až 79 %) je považována za "neutrální" Apo E genotyp
- alela E4 (s četností necelých 14 %) se podílí na ateroskleróze, ovlivňuje patogenezi Alzheimerovy nemoci (ale není nutnou podmínkou jejího vzniku), zrychluje progresi

onemocnění u roztroušené sklerózy. Homozygoti E4/E4 mají 8x vyšší relativní riziko vzniku AN a nižší věk počátku příznaků ve srovnání s homozygoty E3/E3. ApoE4 homozygotů jsou v naší populaci asi 2 %. Vzhledem k tomu, že výskyt ApoE4 je sice spojen s několikanásobně vyšším rizikem rozvoje pozdní formy AN, přitom ale není přímou příčinou AN (tedy kauzální mutací), nedoporučuje se stanovování genotypu ApoE4 v rutinní klinické praxi.

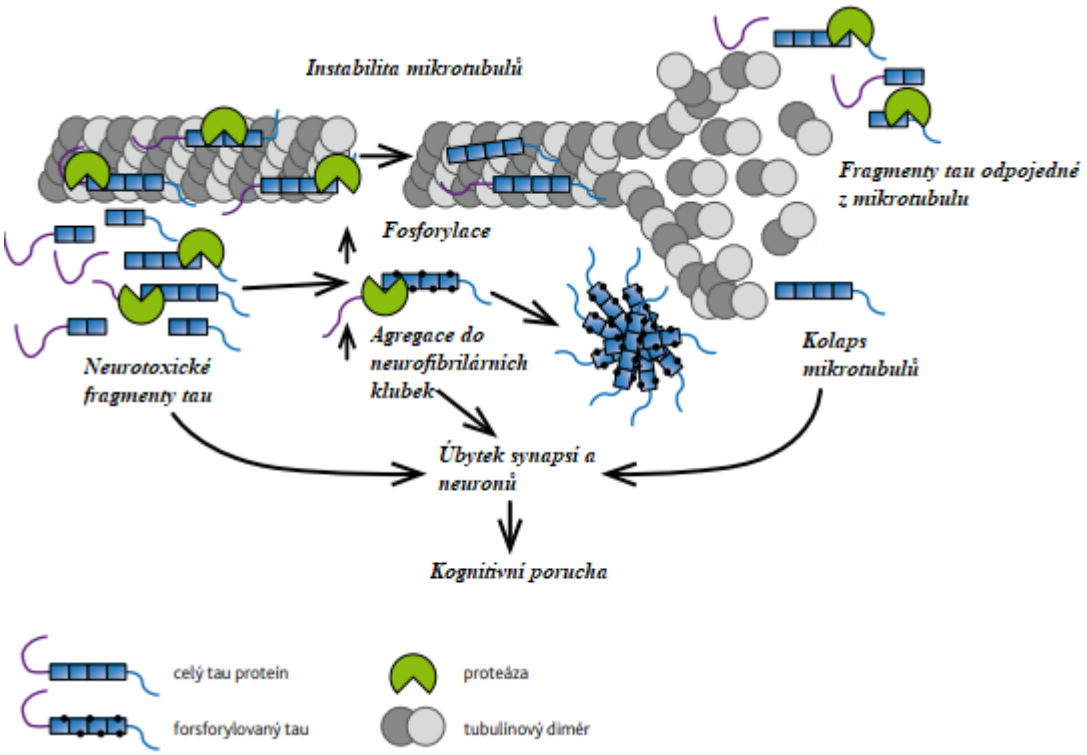
6. FRONTOTEMPORÁLNÍ LOBÁRNÍ DEGENERACE (FTLD)

Společným rysem FTLD je progresivní degenerace frontálních a temporálních laloků mozku, často bývá postižena parietální kůra a bazální ganglia. Podkladem těchto onemocnění jsou poruchy metabolismu některých klíčových proteinů. Abnormálně fosforylovaný tau protein interaguje s metabolickými drahami neuronů a vede k jejich zániku cestou programované buněčné smrti. Vytváří se depozita ve formě inkluzí, která jsou špatně odbouratelná (73). Tato onemocnění jsou velmi heterogenní skupinou, kterou můžeme klinicky rozdělit na 4 skupiny: a) bvFTLD (behaviorální varianta frontotemporální demence), b) PPA (primární progresivní afázie), c) kombinace demence s poruch hybnosti: s extrapyramidovými projevy a s onemocněním motorického neuronu, d) nové neurodegenerace ze spektra FTLD (74). Z neuropatologického pohledu je pro toto onemocnění typické chybění specifických alzheimerovských změn (senilních plak a neurofirilárních klubek). Etiopatologicky podle charakteristických a specifických intraneuronových depozit patologicky změněného proteinu lze FTLD rozdělit na 2 základní skupiny: tauopatie a non-tau-FTLD.

6.1. TAUOPATIE

Tauopatie jsou zapříčiněny abnormálním metabolismem tau proteinu a jeho intraneuronovým ukládáním. Mezi tauopatie řadíme Pickovu nemoc (frontotemporální demence s Pickovými tělísky), kortikobazální degeneraci (CBD), progresivní supranukleární obrnu (PSP) nebo nemoc s argyrofilními zrny (AGD) a některé formy primární progresivní afázie (75). Neuropatologické nálezy s přítomností tau neurofibrilárních klubek avšak bez přítomnosti depozit beta-amyloidu, se označují jako PART (primární na věk vázaná tauopatie). Tyto změny nalézáme také u straších zdravých jedinců. Hromadění abnormálně hyperfosforylovaného tau proteinu v astrocytech je projevem stárnutí mozku. Jako ARTAG (věkově vázaná astrogliopatie s depozity tau proteinu) se označuje morfologické spektrum astrogliální neuropatologie detekované pomocí specifických protilátek namířených proti proteinu tau. Cytomorfologické obrazy se liší od astrogliální patologie primárních primárních tauopatií, jako je PSP, CBD a další. Klinický korelát u nových tauopatií (PART, AGTAG, GGT) (globulární gliová tauopatie) není ještě zcela známý, nicméně byly posány klinické manifestace kombinující demenci, afázii, parkinsonismus, poruchy chování i osobnosti (76).

Obrázek č. 5. Patologie vzniku tauopatií (Necpál J., 2019)



6.2. FTLD-NON TAU

FTLD bez inkluzí tau proteinu (tzv. tau-negativní FTLD) se vyznačují přítomností patologických inkluzí obsahující depozita různých bílkovin mimo tau protein. Pozitivita imunohistochemické reakce s protilátkou proti ubikvitinu u mnoha případů tau negativních frontotemporálních demencí, je souhrnně označována jako „ubikvitinopatie“ (FTLD-U). Byly popsány rodiny s poruchou genů, které mají zásadní vliv na metabolismus proteinu TDP-43. Mezi nejdůležitější patří vlastní gen pro protein TDP-43, tedy *TADRBP*. Byly popsány také mutace v genu pro granulín, ale známé jsou i mutace v genu *VCP* a v dalších genech. V poslední době se také ukazuje důležitá souvislost s expanzí 72 otevřeného čtecího rámce na chromosomu 9, který je mimo jiné důležitý i pro familiární formy FTLD a MND, přičemž byl také zaznamenám i u sporadických forem ALS. FTLD s tau-negativními, ale ubikvitin a TDP-43 (Transactive response DNA binding Protein 43 kD) pozitivními inkluzemi je označována jako FTLD-TDP, varianta s akumulací tzv. fusion in sarcoma proteinu nese označení FTLD-FUS. Klinický obraz FTLD-FUS je charakterizován časným nástupem a poměrně rychlou progresí bvFTD s pyramidovou a extrapyramidovou symptomatikou. Vzhledem k tomu, že postižení je podobně jako u všech dalších FTD asymetrické, může klinický obraz odpovídat i kortikobazálnímu syndromu CBS (77).

FTLD-UPS je poslední varianta FTLD charakterizovaná přítomností ubikvitin pozitivních, TDP-43 a FUS negativních inkluzí. V současné době byla v souvislosti s tímto histopatologickým nálezem popsána pouze jedna klinická jednotka. Jedná se o autozomálně dominantně dědičné onemocnění způsobené mutací *CHMP2B* genu lokalizovaného na 3. chromozomu (49). Klinický obraz odpovídá bvFTD, která může být doprovázena parkinsonismem, dystonií, pyramidovými příznaky nebo myoklonem.

Manifestace non-tau FTLD zahrnuje i jeden ze syndromů progresivní afázie až po asociaci ALS-MND (78).

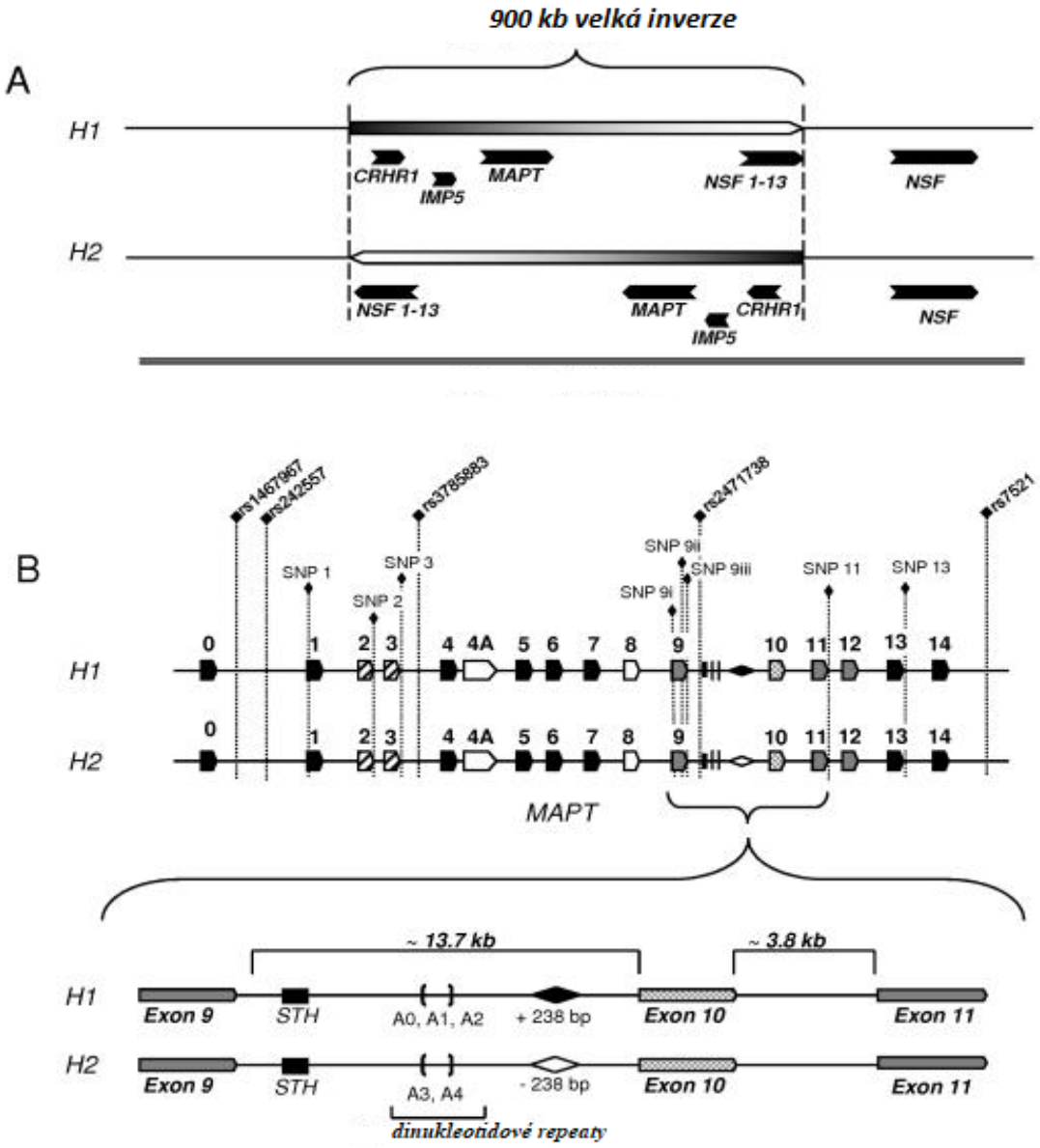
6.3. GENETIKA FRONTOTEMPORÁLNÍ LOBÁRNÍ DEGENERACE

K nejčastějším dědičným formám tauopatií patří mutace v genu *MAPT* (gen pro tau protein). Ve skupině FTLD-non tau je genů více, jde o geny kódující proteiny, které se podílejí na metabolismu a funkci zejména proteinu TDP-43 (gen *TARDBP), GRN* (kóduje protein granulin), genu *C9orf72* (kóduje protein, který hraje důležitou roli v regulaci endosomálního přenosu). Vzácné jsou mutace lokalizované v genech *CHMP2B, FUS, SOD1* (79).

6.3.1. Gen MAPT

Nejběžnějším proteinem patologicky změněným u tauopatií je protein asociovaný s mikrotubuly - tau, který je produktem alternativního sestřihu genu *MAPT.* Frekvence *MAPT* mutací u tauopatií je celosvětově v některých pramenech odhadována až na 50 %. Penetrance je uváděná jako 100%. Jeho transkript prochází komplexním regulovaným sestřihem, jehož výsledkem je několik typů mRNA. Izoformy se liší přítomností nebo absencí 5 z 15 exonů. *MAPT* transkripty jsou exprimovány v nervovém systému v závislosti na stavu neuronálního zrání a typu neuronů (80). Některé polymorfizmy v *MAPT* genu jsou v kompletní vazebné nerovnováze a dědí se jako dva haplotypy, H1 a H2. U převládajícího haplotypu H1 je prokázána asociace s progresivní supranukleární obrnou, kortikobazální degenerací, frontotemporální demencí (FTD). Haplotyp H2 je asociován s familiární frontotemporální demencí (81).

Obrázek č. 6. Grafické znázornění haplotypu H1 a H1



(*A) Lokus MAPT-rozdělen na dva hlavní haplotypy, H1 a H2. Existuje 900 kb velká inverze haplotypu H2 vzhledem k haplotypu H1, pokrývající oblast obsahující MAPT, IMP5, CRHR1 a NSF.*

(B) Sub-haplotypy H1 jsou identifikovány pomocí několika SNP (rs1467967, rs24557, rs3875883, rs2471738, rs7521) (Pittman et al. 2004).

6.3.2. Granulin

Granulin je přítomen ve všech tkáních těla. Je nejaktivnější v buňkách, které se rychle dělí (kožní buňky a buňky výstelky gastrointestinálního traktu). Granulin pomáhá regulovat růst, dělení a přežívání těchto buněk. Hraje důležitou roli v raném embryonálním vývoji, v regulaci imunitní odpovědi a hojení ran i tumorogenezi – a zdá se, že má rozhodující roli pro přežití neuronů (82). Všechny mutace *GRN* genu, které jsou vztaženy k frontotemporální demenci, způsobují ztrátu nebo snížení exprese na mutované alele a v důsledku toho snížení množství vytvořeného granulinu. To vede k sníženému odbourávání proteinu TAR DNA-vazebného proteinu (TDP-43) a k jeho ukládání v mozkových buňkách. TDP-43 agregáty ovlivňují funkci buněk a vedou k neodvratné buněčné smrti neuronů především v oblasti frontálních a temporálních laloků. Frontotemporální demence vázaná na *GRN* představuje asi 5 % všech FTD (a 20% FTD s pozitivní rodinnou anamnézou) (83). Dědičnost onemocnění je autozomálně dominantní, nicméně neexistuje jednoznačná korelace genotypu a fenotypu.

6.3.3. C9orf72

Mutace v *C9orf72* je považována za druhou nejčastější mutací asociovanou s FTLD s frekvencí 14-18 % u familiárních forem. V oblasti mezi prvním a druhým exonem genu *C9orf72* je přítomna repetitivní polymorfní oblast hexanukleotidové sekvence GGGGCC, která se může opakovat od 2-20 kopií až do 700-1600 kopií (84). Patologická expanze GGGGCC v genu *C9orf72* se vyskytuje u některých pacientů s amyotrofickou laterální sklerózou a demencí (frontotemporální lobární degenerace s onemocněním motorického neuronu – FTLD-MND) (85). Pacienti s *C9orf72* expanzí vykazují nižší věk nástupu příznaků, kratší přežití, nástup bulbárních symptomů a nápadnou náchylnost k psychózám a halucinacím.

6.3.4. TDP-43

Protein 43 vázající TAR DNA (TDP-43) je hlavní patologický protein sporadické a familiární frontotemporální lobární degenerace s ubikvitin-pozitivními, tau-negativními inkluzemi (FTLD-U). Podle morfologie a distribuce inkluzí proteinu TDP-43 jsou v rámci tzv. Harmonizované klasifikace FTLD-TDP rozlišovány další čtyři subtypy (typ A, B, C, D) (86) . Různé subtypy FTLD-TDP jsou častěji asociované s různými genovými aberacemi. Většina mutací v *TARDBP* způsobuje, ze TDP-43 je shlukován v C-koncové oblasti (C-teminal domain (CTD). CTD doména TDP-43 nejrelevantnější oblastí proteinu pro jeho agregaci a mutace v této oblasti (např. G294V, G294A a G295S) podporují zvýšenou agregaci proteinu.

7. ONEMOCNĚNÍ S OPAKOVÁNÍM TRIPLETŮ

V současné době je známo asi 10 polyQ onemocnění, pro něž je typická přítomnost patologické expanze trinukleotidů CAG (cytosin – adenin – guanin) nebo CAA (cytosin – adenin – adenin) kódující aminokyselinu glutamin (zkratka Gln či Q – proto „polyQ nemoci“)(tabulka č.2). Výsledkem je změněný protein, který se pak ukládá v neuronu a spouští apoptické mechanismy vedoucí k zániku neuronu. Zmnožení trinukleotidů CCG (cytosin – cytosin – guanin) či CGG (cytosin – guanin – guanin) a další, které nekódují glutamin, se souborně nazývají „non-polyQ nemoci (tabulka č.3). Expandovaný úsek patologicky změněného proteinu následně ovlivní sekundární, terciární i kvartérní strukturu proteinu a vytváří agregáty s charakteristikou amyloidu. Tyto tzv. tripletové choroby mají několik významných podobností: počet tripletů se může mezigeneračně měnit (nestabilita) a v další generaci, která nese mutaci, se patologické expanze prodlužují (anticipace). Platí přitom obecně, že čím více je přítomno tripletů, tím časnější bývá nástup nemoci a zvyšuje se její závažnost (1).

Tabulka č.2. PolyQ nemoci a jejich klíčové proteiny

Nemoc	Protein	Funkce proteinu
SCA1	ataxin-1	regulace transkripce
SCA2	ataxin-2	metabolismus RNA
SCA3	ataxin-3	deubikvitinační enzym
SCA6	ataxin-6	podjednotka napětím řízeného kalciového kanálu
SCA7	ataxin-7	regulace genové exprese
SCA17	TBP	regulace transkripce
DRPLA	atrofin-1	regulace transkripce
SBMA	androgenový receptor	receptor pro testosteron
HN	huntingtin	regulace genové exprese, regulace proteostázy
HDL2	junktofilin-3	součást junkčního komplexu

DRPLA *– dentato-rubro-palido-luysiánská atrofie,* ***HN*** *– Huntingtonova choroba,* ***HDL2*** *– Huntington disease-like 2,* ***SBMA*** *– spinobulbární muskulární atrofie,* ***SCA*** *– spinocerebelární ataxie.*

Tabulka č.3. Non polyQ nemoci a jejich klíčové proteiny

Nemoc	Protein	Funkce proteinu
FRDA	frataxin	homeostáza mitochondriálního železa
FRAXA	FMR1	porucha methylace
FRAXE	AFF2	porucha methylace
FXTAS	FMR1	abnormální zvýšení exprese
SCA8	SCA8	poruchy metabolismu RNA
SCA12	PPP2R2B	regulace transkripce
DM1	DMPK	poruchy metabolismu RNA

FRDA *– Friedreichova ataxie,* ***FRAXA*** *– syndrom fragilního chromosomu X,* ***FRAXE*** *- syndrom fragilního chromosomu X s mentální retardací,* ***FXTAS*** *- syndrom fragilního chromosomu X s tremorem/ataxií,* ***SCA*** *- spinocerebelární ataxie,* ***DM1*** *– myotonická dystrofie typu 1.*

Onemocnění s opakováním tripletů jsou klinicky i neuropatologicky dosti heterogenní a u některých jsou změny a klinický obraz velmi proměnlivé. Podle převládajícího klinického obrazu lze zejména polyQ onemocnění rozdělit do 3 základních skupin:

1. onemocnění projevující se spinocerebelární symptomatikou : spinocerebelární ataxií (SCA1-3, SCA6, SCA7), dentato-rubro palido-luysiánská atrofie a autosomálně recesivní Friedreichova ataxie
2. onemocnění motorického neuron: X-vázaná spinální bulbární muskulární atrofie, Kennedyho nemoc
3. striatokortikální degenerace: HN a vzácná Huntington disease-like 2.

7.1. NEUROPATOLOGIE ONEMOCNĚNÍ S OPAKOVÁNÍM TRIPLETŮ

Pro SCA1, SCA2 a SCA7 je typická olivopontocerebelární atrofie, u SCA3 převládá atrofie pontu, zatímco u i SCA6 a SCA17 převládá atrofie kůry mozečku. U FRDA je nápadná atrofie vestibulo- a spinocerebelárních drah a degenerace zadních provazců míšních. Úbytek motorických neuronů předních rohů míšních a jader hlavových nervů je typický u SBMA. Ve všech polyQ nacházíme intracelulární inkluze obsahující polyQ agregáty postižených proteinů. Ty lze znázornit pomocí nespecifické protilátky proti polyQ sekvenci nebo specifickými protilátkami proti patologicky změněnému proteinu (1).

7.2. GENETIKA ONEMOCNĚNÍ S OPAKOVÁNÍM TRIPLETŮ

Podstatou mutace podmiňující vznik onemocnění je zmnožení repetic. U HN dochází ke zmnožení tripletu CAG v genu *HTT* kódujícím protein huntingtin. Zmnožení glutaminu mění

strukturu a konfirmaci huntingtinu a vzniklá proteinopatie svými důsledky přímo podmiňuje patogenezi nemoci. HN je autosomálně dominantní, s vysokou mírou penetrance. Při ≥40 tripletech jedinec zcela jistě onemocní HN (87). U tripletů s nižším počtem (36-49) je prognóza nejistá. Tripletová expanze GAA u FRDA v 1. intronu genu pro mitochondriální protein frataxin (*FXN, X25, FRD*) je důsledkem tzv. loss of function mutace, tedy ztráta funkce proteinu, zejména v rámci metabolismu železa. U zdravých jedinců je počet opakování GAA <34, ale je také popsáno rozmezí 70-1700. Délka expanze tripletů inverzně koreluje s věkem nástupu obtíží, závažností onemocnění a přítomností systémových komplikací (88) (1).

8. OSTATNÍ NEURODEGENERACE

Navzdory molekulárně biologických, biochemických pokroků stále ještě existuje skupina nezařazených neurodegenerací. Jsou to většinou vzácné nemoci s ojedinělými popisy v literatuře.

8.1. DEMENCE BEZ HISTOLOGICKÉHO KORELÁTU (DLDH)

U těchto demencí nebyl nalezen biochemický proteinový korelát a nejsou přítomné žádné jednoznačné neuropatologické ani klinické diagnostické charakteristiky. Tato skupina onemocnění se výrazně umenšila objevem proteinu TDP-43 a jeho úlohy ve skupině frontotemporálních lobárních degenerací (89) .

8.2. DĚDIČNÉ AMYLOIDÓZY

Familiární britská demence (FBD) a familiární dánská demence (FDD) jsou asociovány s patologicky změněným proteinem *BRI2,* kdy se z prekurzorového proteinu stává malformovaný peptid ABri v případě FBD a ADan u FDD. Jedná se o autosomálně dominantně dědičná onemocnění s demencí, spasticitou, kataraktou, ataxií a hluchotou. Neuropatologický nález zahrnuje masivní cerebrální amyloidovou angiopatií s depozity amyloidu ve formě plak i četných neurofibrilárních klubek (90).

8.3. NEURODEGENERACE S AKUMULACÍ ŽELEZITÝCH SOLÍ V MOZKU

Nadměrná akumulace kovů v nervovém systému může být toxická, může vyvolávat oxidační stres, narušovat mitochondriální funkce a zhoršovat aktivitu mnoha enzymů. Klinické studie prokázaly silnou korelaci mezi expozicí aberantních kovů a řadou neurologických onemocnění, včetně Alzheimerovy choroby, amyotrofické laterální sklerózy, poruch autistického spektra, Guillainovy-Barrého choroby, Huntingtonovy choroby, roztroušené sklerózy, Parkinsonovy choroby a Wilsonovy nemoci (91). Hlavní příčinou oxidační toxicity z přechodných kovů je tvorba volných kyslíkových radikálů - ROS (Reactive Oxygen Species), nejdůležitějšího oxidačního činidla v buňkách. Kovy podílející se na vzniku onemocnění jsou převážně železo, měď, zinek a mangan. NBIA (neurodegeneration with brain iron accumulation) je skupina vzácných genetických onemocnění se zvýšenou akumulací železitých substancí v bazálních gangliích. Nejčastější mutace jsou popisovány v genech *PKAN, PLAN, MPAN, BPAN* (92). Wilsonova nemoc je vzácné hereditární metabolické onemocnění s autozomálně recesivní dědičností. Příčinou je dysfunkce proteinu ATP7B způsobující poruchu vylučování mědi z hepatocytů do žlučových cest a inkorporaci mědi do ceruloplazminu.

8.3.1. Molekulární podstata toxicity kovů

Kovová dyshomeostáza je škodlivá pro lidské buňky. Intracelulární a extracelulární hladiny kovů jsou přísně regulovány prostřednictvím složité sítě. Nadměrná koncentrace solí kovů může způsobit buněčnou toxicitu a patologické poškození. Kromě změny membránového potenciálu, zejména v neuronech, se kovové ionty mohou vázat a ovlivňovat aktivitu proteinů / enzymů a nukleových kyselin, což může způsobit cytotoxicitu. Těžké kovy, jako je kadmium a olovo mohou způsobit depolarizaci membrány tím, že zablokují přítok iontů vápníku do buňky (93).

9. OBECNÉ ASPEKTY MOLEKULÁRNĚ-GENETICKÉ DIAGNOSTIKY NEURODEGENERATIVNÍCH ONEMOCNĚNÍ

Vyšetření by mělo být indikováno lékařským genetikem. Nejvýznamnějším klinickým vodítkem, které vede k podezření na hereditární neuropatii je familiární výskyt, zejména u forem s autosomálně dominantní (AD) dědičností. U forem s autosomálně recesivní (AR) dědičností, ale také u AD forem v případě vzniku mutace *de novo* jde spíše o sporadický původ. Také je důležité vědět, že různé mutace v jednom genu mohou vyvolávat řadu velmi odlišných fenotypů. V případě relevantního podezření na hereditární původ neuropatie je další praktickou otázkou, jakou zvolit strategii při výběru genetických testů. Kromě metod zaměřených na cílový gen je v současnosti k dispozici řada metod pokročilého genetického testování počínaje panely genů zaměřených na specifický fenotyp a umožňujících souběžné testování celé skupiny nejvýznamnějších relevantních genů až po celoexomové či celogenomové sekvenování (94). I přes vysokou nákladnost může být jejich použití ekonomické, pokud je správně indikováno. Při tomto rozhodování musíme brát v úvahu prevalenci výskytu mutací určitých známých genů u definovaných subtypů hereditárních neuropatií. Pokud je testování frekventních mutací negativní a fenotyp silně podporuje podezření na hereditární neuropatii a neobsahuje žádná specifická vodítka, je nutné zvážit použití genových panelů/chipů a metody pokročilého sekvenování. Vzhledem k vysoké nákladnosti a náročnosti těchto metod je vhodné, aby je indikoval specialista se zkušeností v oblasti hereditárních neuromuskulárních chorob po konzultaci s genetikem (95).

10. PŘÍLEŽITOST K TERAPII

Požadavek léčit příčinu choroby splňuje až genová terapie. Je-li nemoc způsobena nefunkční nebo chybějící bílkovinou, je teoreticky možné dodat tělu genetický materiál pro její tvorbu – buď holou DNA, nebo úsek DNA navázaný na nosič (vektor). Tím je většinou geneticky upravený vir. Komplexní přístup představuje implantace umělé geneticky naprogramované tkáně (neoorgánu), která by syntetizovala požadovanou bílkovinu. V současnosti probíhá výzkum genové terapie Alzheimerovy demence a Parkinsonovy choroby. *Antisense drugs* a genová terapie by se v některých indikacích mohly kombinovat. V posledním desetiletí výzkum neurodegenerativních chorob zaznamenal výrazný pokrok. Genetické a biologické modely těchto chorob představují nové možnosti terapie a prevence. Dosavadní léčba byla založena na regulaci neurotransmiterů a nespecifickém zlepšení metabolizmu neuronů. Na obzoru jsou však léčebné postupy, které by již mohly postihovat samotnou podstatu těchto vážných chorob (96).

Doposud neexistuje účinná léčba ALS. Od roku 1995 je antiglutamatergická látka riluzol jedinou farmakologickou léčbou podávanou pacientům s ALS, se střední dobou přežití až 3 měsíce (97). Americká FDA schválila edaravon jako lék ALS po úspěšných klinických studiích v Japonsku a Jižní Koreji (98). Edaravon je neuroprotektivní léčivo, které působí jako antioxidant. Léčba snížila funkční pokles po dobu 6 měsíců, když byla zahájena na počátku progrese onemocnění (99). Přesto tato dvě ALS léčiva mají jen omezené příznivé účinky a není k dispozici žádný lék, který významně prodlužuje životnost pacientů s ALS, což naznačuje, že je naléhavě nutná lepší léčba. Rychlý vývoj v genetických studiích identifikujících nové geny ALS a související dráhy onemocnění slibuje nové terapeutické strategie. Například cílení na geny ALS, genetické modifikátory nebo příbuzné molekuly onemocnění antisense oligonukleotidy (ASO) vykázaly slibné výsledky. Ve fázi I klinického hodnocení bylo přímé podání ASO proti *SOD1* do CSF pacientů s fALS intratekální infuzí schopno eliminovat mutantní *SOD1* bez nežádoucích účinků (100). V souladu s těmito nálezy léčba ASO proti *SOD1* zlepšila přežití lidského fALS indukovanými pluripotentními kmenovými buňkami (iPSC) a snížila expresi apoptotických markerů. Kromě kauzálních genů byly zaměřeny také genetické modifikátory. Rozšířené polyglutaminové repetice v *ATXN2* způsobují spinocerebelární ataxii typu 2, ale střední repetice byly spojeny se zvýšeným rizikem ALS (101). *ATXN2* tvoří komplex s TDP-43 a je silným modifikátorem toxicity TDP-43 ve zvířecích a buněčných modelech (102) (103) (96).

11. CÍLE A HYPOTÉZY PRÁCE

11.1. CÍL PRÁCE

Běžná neurodegenerativní onemocnění, jako je Alzheimerova choroba, Parkinsonova choroba, amyotrofická laterální skleróza, frontotemporální lobární degenerace a Huntingtonova choroba jsou oslabující poruchy s rostoucí prevalencí v moderních společnostech stárnutí. Desetiletí výzkumu jednotlivých nemocí prostřednictvím různých přístupů nabízí hluboký pohled do fenotypových projevů a molekulárních základů každé nemoci. Je zajímavé, že byly pozorovány vzorce konvergujících funkcí napříč neurodegenerativními chorobami, jako je demence u AN, PN a ALS vyzývající k hledání lepšího pochopení vztahů mezi neurodegenerativními chorobami za účelem odhalení mechanismů specifických pro dané onemocnění i potenciálně sdílených mechanismů.

V této práci jsme se snažili o systematické studium a hodnocení nálezů pacientů s neurodegenerativním onemocněním, identifikovat geny a rizikové lokusy, provést screening genetických faktorů podílejících se na neurodegenerativních onemocněních jako jsou AN, FTD, ALS nebo PD a odhalit fenotypové překrývání mezi sporadickými nebo familiárními neurodegenerativními formami demence pomocí genetického pozadí.

Cílem této práce bylo:

1) MUTAČNÍ SCREENING GENŮ U PACIENTŮ SE sCJD SAMOSTATNĚ A sCJD S DALŠÍ PROTEINOPATIÍ

2) FENOTYPOVÉ A GENOTYPOVÉ POROVNÁNÍ VZÁCNÝCH PŘÍPADŮ GSS ZACHYCENÝCH V ČESKÉ REPUBLICE

3) KOMPLEXNÍ ANALÝZA GENŮ ASOCIOVANÝCH S ALS/FTLD

4) ANALÝZA A MAPOVÁNÍ VARIANT GENU TIA1 ASOCIOVANÝCH S ALS/FTLD

11.2. HYPOTÉZY

1) Analýza proteomu může přinést důležitější informace o buněčném chování než profilování celého transkriptomu mezi neurodegenerativními chorobami.

2) Monogenně vázané neurodegenerace mohou mít atypické klinické prezentace.

3) Příčinou souběžného neurodegenerativního onemocnění může být souběžná atypická prezentace onemocnění.

12. EXPERIMENTÁLNÍ ČÁST - MATERIÁL A METODIKA

Analýza genů probíhala na úrovni genomové DNA s předpokladem vlivu na proteinovou sekvenci. Byla hodnocena pouze kódující část genu a přilehlé intronové oblasti. Genetická analýza genů byla prováděná jednak z autopticky získaných vzorků definitivně potvrzených případů a jednak od žijících pacientů sledovaných v kognitivních centrech. DNA byla izolovaná z kostní dřeně nebo z periferní krve (QIAamp DNA Kits) na Ústavu patologie a molekulární medicíny 3. LF UK a FTN v laboratoři molekulární genetiky. U všech vzorků, které byly neuropatologicky ověřené nebo na základě genetického a neurologického vyšetření, jsme prováděli systematickou analýzu genů souvisejících s nejdůležitějšími neurodegenerativními chorobami - AN, FTD, ALS, CJD a sCJD v komorbiditě s AN a PART. Kromě toho jsme porovnávali haplotypy genu *MAPT* (mikrotubuly asociované s proteinem tau) mezi pacienty s sCJD a pacienty s sCJD a PART nebo sCJD a AN. Poté jsme studovali interakci mezi genem Apolipoprotein E (*APOE*) a *PRNP* u pacientů s sCJD.

Pitva mozku i míchy byla provedena u všech pacientů zkušenými neuropatology Ústavu patologie a molekulární medicíny 3. LF UK a Fakultní Thomayerovy nemocnice (zejména prof. Matěj), který provedl také morfometrické skórování immunoreaktivních globulárních struktur, inkluzí podobných neurofibrilárním klubkům (NCIs) cytoplasmatických inkluzí oligodendogrií (GCIs), astrogliálních cytoplasmatických inkluzí (TA) a neurofilních vláken a teček tau proteinu.

Do studie bylo zahrnuto 245 případů s neurodegenerativním onemocněním neprionového typu a 305 případů, u kterých byla neuropatologicky ověřena CJD. Další skupinu tvořily případy s různým typem neurodegenerativního onemocnění, u kterých nebyla provedena neuropatologická verifikace. Tuto skupinu tvořilo 230 případů, kterým byl na základě genetické konzultace indikován genetický screening.

Pitevní nálezy **neprionových onemocnění** ze vzorků mozkových tkání uváděny jako možné / pravděpodobně CJD

Neurodegenerativní onemocnění	Alzheimerova choroba	88
	Frontotemporální demence	66
	Demence s Lewyho tělísky	27
	Progresivní supranukleární obrna	8
	Atrofie více systémů	4
	Kortikobazální degenerace	2

	Parkinsonova choroba	1
Neuroinfekce a autoimunitní onemocnění	Encefalitida	20
	Maligní roztroušená skleróza (varianta Marburg)	2
Ischemické a anoxické stavy	Subkortikální vaskulární demence	7
	Postanoxická encefalopatie	7
Tumor	Primární CNS lymfom	5
	Gliomatosis cerebri	1
	Meningeální karcinomatóza	1
	Metastatický karcinom	1
Metabolická encefalopatie	Wernicke-Korsakoff	5
Ostatní	Subdurální hematom	1

Neuropatologicky **potvrzené případy TSE**

rok	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	Total
sCJD	13	12	18	17	21	17	16	21	18	15	167
fCJD	1	0	3	1	0	6	2	0	1	1	15
GSS	0	2	0	1	2	0	0	1	0	1	7
											190
								Celkový počet			**305**

Příbuzní podepsali s testováním informovaný souhlas schválený multicentrickou etickou komisí Institutu klinické a experimentální medicíny a Fakultní Thomayerovy nemocnice (pořadové číslo: G21-12).

12.1. CÍLENÉ GENY PRO SANGEROVO SEKVENOVÁNÍ

Pro genetickou analýzu byly vybrány geny, které byly nejvíce asociovány s neuropatologickou a neurologickou diagnózou.

Gene	**Associated phenotype**	**Locus (GRCh38.p12)**
APP	AD	chr21:25,880,550-26,170,770
PSEN1	AN	chr14:73,136,507-73,223,691

PSEN2	AN	chr1: 226,870,616-226,896,098
APOE	AN	chr19: 44,905,796-44,909,393
PRNP	Prion D	chr20: 4,686,350-4,701,590
GRN	FTD	chr17: 44,345,246-44,353,106
C9orf72	FTD-ALS	chr9: 27,546,546-27,573,481
MAPT	FTD	chr17: 45,894,554-46,028,334
TARDBP	FTD-ALS	chr1: 11,012,344-11,025,739
FUS	ALS	chr16: 31,180,139-31,191,605
SOD1	ALS	chr21: 31,659,666-31,668,931
FTL	FTD	chr19:48,965,308-48,966,878

12.2. CÍLENÝ PANEL PRO NGS

Cílený panel TruSeq Neurodegeneration Panel s širokým pokrytím 118 genů spojených s hlavními neurodegenerativními chorobami. Obsahuje více než 8,7 MB oblastí, včetně regulačních oblastí, exonů, intronů, nepřekládaných oblastí (UTR) a promotorů. Geny zahrnuté do panelu Neurodegeneration Sequencing Panel TruSeq pokrývají nejčastější typy neurodegenerativního onemocnění (• Alzheimerova choroba • Parkinsonova choroba • Amyotrofická laterální skleróza • Frontotemporální demence • Demence s Lewyho tělísky • Dystonie • EarlyOnsetDementia).

12.2.1. Genetický screening

Cílený panel zachytil všechny exony genů a přilehlé intronové oblasti, aby byla pokryta místa sestřihu. Primery byly navrženy pomocí softwaru mPCR pomocí technologie amplicon target amplification technology (Agilent, https://www.agilent.com). K přečištění PCR produktu před vlastní sekvenační reakcí jsme použili enzymatickou směs se SAP – shrim alkaline phosphatase (Fermentas, St. Leon-Rot, Německo) a EXO – exonuclease I (Fermentas, St. Leon-Rot, Německo), která se používá pro specifické a vysoce koncentrované produkty v poměru 1:0,5. Sekvenační reakce jsme připravili z RR mixu (Applied Biosystems, Foster City, USA) a odpovídajícího sekvenačního primeru v poměru 2 : 1 µl pro příslušný počet vzorků. Kapilární elektroforéza byla na genetickém analyzátoru Applied Biosystems GA 3130. Vyhodnocení dat bylo provedeno pomocí softwaru Sequencing Analysis.

Pro NGS byly specifické cílové oblasti amplifikovány pomocí multiplexní PCR, následně purifikované pomocí ekvimolárně sdružených amplikonů pomocí kuliček Agencourt AMPureXP (Beckman Coulter, CA, USA). Jednotlivé čárové kódy (Illumina Nextera XT) byly začleněny do kroku univerzální PCR před poolováním vzorků. Knihovny byly sekvenovány na

platformě MiSeq pomocí reagenční soupravy v3 s délkou čtení spárovaného konce 300 bp (Illumina, San Diego, CA, USA). Byly hodnoceny nonsense, splice site, indel a missense mutace s alelovou frekvencí menší (MAF) ≤ 1%.

12.3. STATISTICKÉ METODY

Ke zpracování získaných dat byly použity statistické a prediktivní nástroje zejména. Účinky vzácných (MAF ≤ 1%) missense variant na strukturu a funkci proteinu byly předpovězeny pomocí SIFT (http://sift.jcvi.org/), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) a SNP & GO (https://snps-and-go.biocomp.unibo.it/snps-and-go/).

13. EXPERIMENTÁLNÍ ČÁST – VÝSLEDKY

13.1. MUTAČNÍ SCREENING GENŮ U PACIANTŮ S SCJD A SCJD S DALŠÍ PROTEINOPATIÍ

Eva Parobkova, Julie van der Zee, Lubina Dillen, Christine Van Broeckhoven, Robert Rusina, Radoslav Matěj. **Sporadic Creutzfeldt-Jakob disease and other proteinopathies in comorbidity.**

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Sporadic Creutzfeldt-Jakob Disease and Other Proteinopathies in Comorbidity

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Background: Sporadic Creutzfeldt–Jakob disease (sCJD) is the most common type of a group of transmissible spongiform encephalopathies (prion diseases). The etiology of the sporadic form of CJD is still unclear. sCJD can occur in combination with other neurodegenerative diseases, which further complicates the diagnosis. Alzheimer's disease (AD), e.g., is often seen in conjunction with sCJD.

Method: In this study, we performed a systematic analysis of 15 genes related to the most important neurodegenerative diseases - AD, frontotemporal dementia, amyotrophic lateral sclerosis, prion disease, and Parkinson's disease - in a cohort of sCJD and sCJD in comorbidity with AD and primary age-related proteinopathy (PART). A total of 30 neuropathologically verified cases of sCJD with and without additional proteinopathies were included in the study. In addition, we compared microtubule-associated protein tau *(MAPT)* haplotypes between sCJD patients and patients with sCJD and PART or sCJD and AD. Then we studied the interaction between the Apolipoprotein E gene (*APOE*) and *PRNP* in sCJD patients.

Results: We did not find any causal mutations in the neurodegenerative disease genes. We did detect a p.E318G missense variant of uncertain significance (VUS) in *PSEN1* in three patients. In *PRNP*, we also found a previously described non-pathogenic insertion (p.P84_Q91Q).

Conclusion: Our pilot study failed to find any critical differences between pure sCJD and sCJD in conjunction with other comorbid neurodegenerative diseases. Further investigations are needed to better understand this phenomenon.

Keywords: Creutzfeldt-Jakob disease, Alzheimer's disease, β amyloid, tau protein, neurodegenerative disease

INTRODUCTION

Neurodegenerative diseases are characterized by intra-or extracellular accumulation of specific protein aggregates in the central nervous system (CNS) (1). These proteins have a predominantly β-sheet form and are found in a number of neurodegenerative diseases such as Alzheimer's disease (AD); synucleinopathies (Parkinson's disease (PD), multiple system atrophy, dementia with Lewy bodies); transmissible spongiform encephalopathies (TSE; also known as prion disease); amyotrophic lateral sclerosis and frontotemporal dementia (2). There is a significant overlap of symptoms resulting from the multiplication and tissue storage of protein aggregates in the brain, leading to progressive neuronal dysfunction and neurodegeneration (3, 4).

Creutzfeldt-Jakob disease (CJD; MIM #176640), the most common human prion disease with an estimated incidence of 2 cases per million per year, is comprised of several clinical-pathological phenotypes and occurs in four unique forms (sporadic, genetic, variant, or acquired), each with seemingly distinct etiologies (5).

CJD can coexist with other neurodegenerative diseases because the presence of both Aβ and tau pathology is not unusual in sporadic and genetic CJD brains (6–9). Primary age-related proteinopathy (PART) is a common pathology involving misfolded tau protein aggregates associated with human aging (10). PART can cause cognitive impairment in the absence of AD (11); additionally, the coexistence of PART and sporadic CJD (sCJD) has been reported (12). A major genetic risk factor for PART is the haplotype of the microtubule-associated protein tau (*MAPT*) (13). The frequency of Apolipoprotein E (APOE) ε4 is much lower in PART, being ∼10% (10, 14), whereas its prevalence in AD exceeds 45% (15, 16). These studies suggest that APOE ε4 allele deficiency – in contrast to AD – is not a risk factor for PART.

Coexistence with other neurodegenerations is relatively common in sCJD patients. Since clinical symptoms of sCJD can overlap with manifestations of other comorbid disorders, establishing a clinical diagnosis in patients with rapidly progressive dementia is very difficult (17), and a definite diagnosis can only be made after a neuropathological examination of the brain.

Our goal was to identify disease-associated variants using genetic studies of sCJD patients. For this reason, we compared sCJD patients without any comorbid proteinopathies to sCJD patients with AD and sCJD patients with PART.

MATERIALS AND METHODS

Study Population

Our study was designed as a retrospective study. We included patients with post-mortem confirmed sCJD, as well as information regarding clinical presentation and data from neuropsychological testing, biochemical analysis, EEG, and neuroimaging. Neuropathological diagnoses, including prion protein immunoassays, were provided according to standard protocols National CJD Research & Surveillance Unit. Protocol: Surveillance of CJD in the UK) (18) used by the National reference laboratory for human prion diseases at the Department of Pathology and Molecular Medicine, Prague, Czech Republic. Molecular genetic analyses were performed in the Neurodegenerative Brain Disease group of the VIB Center for Molecular Neurology, Antwerp, Belgium.

We divided our cohort into three subgroups: (1) isolated sCJD neuropathology, (2) sCJD and PART or early stage AD (NIA consensus criteria level "low") (19), and (3) sCJD with more advanced AD (NIA consensus criteria level A2 and or higher).

The Molecular Diagnostics Study Group

All autopsied patients (30/30) fulfilled the WHO diagnostic criteria for definite sporadic CJD (18)[1] and were genetically profiled for the most common genes ($n = 15$) associated with AD (*APP, PSEN1, PSEN2, APOE*), the FTD-ALS spectrum (*MAPT, GRN, TARDBP, FUS, SOD1, VCP*), prion disease (*PRNP*), and PD (*LRRK2, PRKN, SNCA*) (**Supplementary Table 1**).

Other available clinical data, which were designated as variables (including age at onset, age at death, gender, and symptoms occurring during the disease), were analyzed to determine how they affected the pathogenesis of sCJD and the concomitant Aβ and tau pathologies.

Genetic Screen

Mutation analyses by gene panel sequencing were performed on genomic DNA extracted from bone marrow. The targeted gene panel captured all exons of the 15 genes and flanking intronic regions to cover the splice sites. Using amplicon target amplification technology (Agilent, https://www.agilent.com), primers were designed using mPCR software (20) (**Supplementary Material**). Specific target regions were amplified using multiplex PCR, followed by purification of the equimolar pooled amplicons using Agencourt AMPureXP beads (Beckman Coulter, CA, USA). Individual barcodes (Illumina Nextera XT) were incorporated in a universal PCR step prior to sample pooling. Libraries were sequenced on a MiSeq platform using the v3 reagent kit with a paired-end read length of 300 bp (Illumina, San Diego, CA, USA). Non-sense, splice site, indel, and missense variants, with a minor allele frequency (MAF) ≤ 1%, were selected.

Results

We analyzed data from 30 patients ($n = 30$) with a mean age at onset (AAO) of 58.4 ± 5 years, and a male-to-female ratio of 18:12. Ten cases had sCJD without any other comorbid proteinopathy, 10 cases had sCJD with tauopathy and/or early evolved AD, and 10 cases had sCJD with more developed AD. Family histories were available in 24 cases (82%), with only one patient (3.4%) having a positive family history for dementia. No family histories of CJD were reported. Effects of rare (MAF ≤ 1%) missense variants on protein structure and function were predicted using SIFT (http://sift.jcvi.org/), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) and

[1]www.cjdsupport.org/, https://www.cjdsupport.org.au/site/wp-content/uploads/2017/04/PRNP-Guidelines-160417.pdf

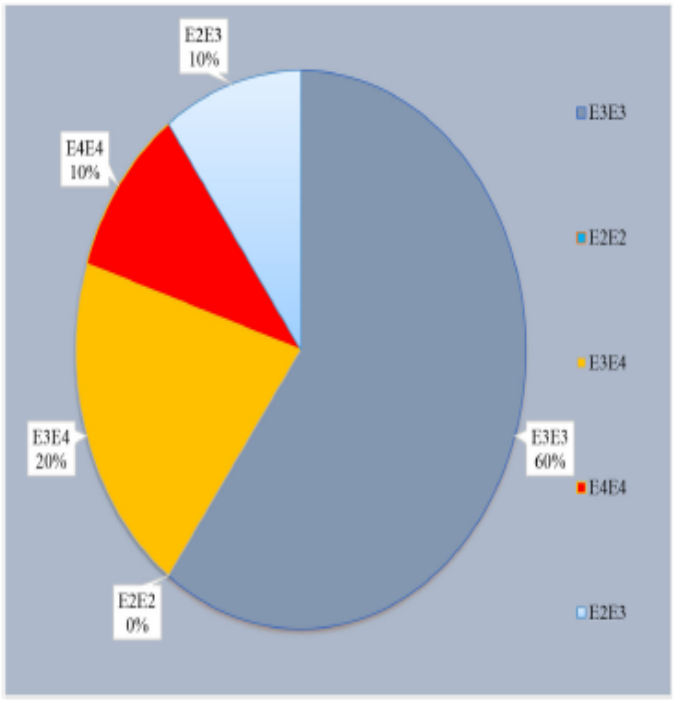

FIGURE 1 | Distribution of *APOE* polymorphism carriers in our cohort ($n = 30$).

SNP&GO (https://snps-and-go.biocomp.unibo.it/snps-and-go/) (**Supplementary Table 2**).

Clinical manifestations included mild to moderate dementia with predominant executive and speech/language impairment (aphasia, dysarthria) with less impaired memory and visuospatial function. Behavioral and psychiatric manifestations (depression, apathy, irritability, anxiety, aggression, visual hallucinations, and insomnia) were described in most patients. Motor symptoms typically included Parkinsonism, spasticity, gait disturbance, and/or immobility (**Supplementary Table 3**).

Mutation Screening

Gene panel screening (15 genes) for variants and mutations associated with AD, FTD-ALS, and PD, revealed 4 rare, protein-modifying variants (**Supplementary Table 2**). Effects on protein structure and function were predicted using SIFT (http://sift.jcvi.org/), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) and SNP&GO (https://snps-and-go.biocomp.unibo.it/snps-and-go/). In *PSEN1*, the p.E318G polymorphism in *PSEN1* was found in three patients (10.3%), of which one had pure sCJD, and two were sCJD + AD. No relevant variants were observed in the other AD genes *APP* and *PSEN2*. In *PRNP*, the p.P84_Q91Q insertion was detected in one patient with β-amyloidopathy. This variant is considered non-pathogenic (21). Furthermore, one benign missense variant was present in *GRN* and one in *SOD1*. We did not find any potential disease-causing mutations in the PD genes *SNCA*, *LRRK2*, and *PRKN*; silent mutations were found (**Supplementary Table 2**).

Genetic Predisposing Factors - ε4 Allele of Apolipoprotein *E* (*APOE*)

APOE polymorphic variants were tested at codons 112 and 158. Of the 30 cases in our study, 10% ($n = 3$) carried the ε4 allele of the *APOE* gene (**Figure 1**). All three cases were AD level A2 (AAO 62, 75, 83 years). The distribution of the polymorphic codon 129 of *PRNP* and *APO* genotypes in sCJD patients are shown in **Supplementary Table 4**. We found no association between *APOE* ε4 allele status and sCJD; however, the *APOE* ε4 was seen in two *PRNP* M129M homozygotes ($n = 2$).

MAPT Haplotype Association With Sporadic CJD

We analyzed MAPT haplotypes in both isolated sCJD cases and in cases with sCJD and tauopathy. We identified only one case with the H2/H2 haplotype, and they were in the comorbid subgroup (**Supplementary Table 5**). As such, our study shows no evidence of an association between MAPT gene variations and sCJD, which could have contributed to the tau deposits in the CNS.

DISCUSSION

In our study of 30 cases of sCJD in the Czech Republic (the annual rate of definite CJD is about 20 cases/yr.), we analyzed the most important genes related to neurodegeneration. The cognitive profile in our patients was characterized by a

heterogeneous manifestation, with predominant involvement of executive and speech/language functions with a significant proportion also having behavioral manifestations (including visual hallucinations).

We did not detect any pathogenic mutations in the *PRNP* gene. Our study also tried to determine if there were any predisposing genetic factors that could account for the occurrence of comorbid Aβ and tau protein deposits in CJD brains. Previous studies have provided evidence that comorbid proteinopathy is not unusual in CJD brains, although the exact mechanism by which β-amyloid and tau deposits spread within brain tissue remains unclear (22). Since several studies have documented a possible spread of β-amyloid in brain tissue (23, 24), we performed a mutation analysis of *APP* (Aβ encoding exons) as well as the coding region of *PSEN1* and *PSEN2*. However, we did not find any mutations in the genes that would explain the increased Aβ42 production.

There is only sparse evidence supporting the potential interaction between *APOE* and *PRNP* in sCJD. Recent studies that analyzed the influence of *APOE* on CJD have yielded discordant results. Three of our cases had the *APOE* ε4 genotype (AAO > 70 years on average), i.e., β-amyloidopathy level A2 and Methionine/Methionine homozygosity at codon 129 of the *PRNP* gene (M129M). Recent studies have suggested variants of PRNP129 (methionine/methionine, methionine/valine, valine/valine) as possible modifiers of AD disease (25). However, because of the small sample size of our study, this interpretation should be approached cautiously. Further studies should be carried out to assess the effects of PRNP129 in the AD phenotype. We found no influence of the *APOE* genotype relative to the age at onset, nor any significant differences in the distribution of the *APOE* ε4 and ε2 genotypes relative to those with isolated sCJD and those with sCJD and AD. Our results are consistent with other studies showing that *APOE* is not a risk factor for CJD (26–29).

The pathology of tau in sCJD brains is not unique, and in our cohort, this additional pathology was seen in 6 of the 30 definite CJD patients (30%) (6). Tau is encoded by the *MAPT* gene, and there are two common *MAPT* extended haplotypes, i.e., H1 and H2 (29). Only one study has investigated the role of MAPT in the etiology of sCJD (30). There is somewhat more evidence regarding the role of MAPT haplotypes (H1 and H2) in neurodegenerative diseases. H1 has been linked to FTLD and AD (31), whereas H2 is associated with a lower risk for developing late-onset AD (32). Our study shows no evidence for any association between *MAPT* gene haplotypes and sCJD.

The coexistence of CJD and PD is exceedingly rare. Several reported case studies show that α-synuclein amyloid deposits in CJD patients are associated with a slower disease course. The precise molecular mechanism explaining how misfolded α-synuclein accumulates and spreads in synucleinopathies is still unknown (33). Sequence or copy number variants in at least six genes (*SNCA, LRRK2, PRKN, PINK1, DJ-1*, and *ATP13A2*) have been identified to cause monogenic forms of PD (34). To date, no mutations responsible for PD have been reported in patients with CJD. Due to the low incidence of patients with proven CJD and PD, it is not clear whether there are gene interactions between CJD and PD. Our study, however, was not focused on the issue of sCJD and synucleinopathy, due to the extremely low incidence of both pathologies in comorbidity. This issue is, nevertheless, a promising direction for future research, and as such, it could help us better understand the genetic background as well as perhaps offer novel therapeutic options.

In conclusion, we failed to find any association between the investigated genes and the accumulation of specific protein aggregates in the examined brain tissue. These findings suggest that comorbid neurodegenerative disorders in sCJD behave as if they were independent processes taking place within the same brain; additionally, the underlying pathophysiology of comorbid protein deposits in CJD appears to have a complex multifactorial origin.

It would, however, be promising in the future to examine other risk genes for AD, FTD, and PD, and their potential association with CJD (**Supplementary Table 6**) (35). The search for genetic evidence of clinical, pathological, and possible molecular overlap between neurodegenerative diseases certainly needs to continue and would be best done with a larger multicenter cohort.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: This manuscript utilizes proprietary data. Requests to access these datasets should be directed to Julie van der Zee, julie.vanderzee@uantwerpen.vib.be, Neurodegenerative Brain Diseases Group, VIB Center for Molecular Neurology, VIB, Antwerp, Belgium. Primers for the targeted assay were designed using proprietary mPCR software from Agilent (previously Multiplicom) (20).

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JZ created the concept and design of the project. CVB provided laboratory facilities for the practical implementation of the entire project, provided complete instrumentation of the VIB Center for Molecular Neurology. JZ and CVB critically revised the manuscript. LD provided recommendations and specific approaches to the samples analysis (analysis tools) as an expert technician in the laboratory. RR diagnosed in detail the neurological cases mentioned in the article. RM verified the neuropathological diagnosis of neurodegenerations

and approved the final version to be published. All authors participated in the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.596108/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary Material

The data analyzed in this study is subject to the following licenses/restrictions: This manuscript utilizes proprietary data. Requests to access these datasets should be directed to julie.vanderzee@uantwerpen.vib.be, Neurodegenerative Brain Diseases Group, VIB Center for Molecular Neurology, VIB, Antwerp, Belgium. Primers for the targeted assay were designed using proprietary mPCR software from Agilent (previously Multiplicom), Goossens et al., 2009.

Table S1: Gene content of the targeted assay

Gene	**Associated phenotype**	**Locus (GRCh38.p12)**
APP	AD	chr21:25,880,550-26,170,770
PSEN1	AD	chr14:73,136,507-73,223,691
PSEN2	AD	chr1: 226,870,616-226,896,098
APOE	AD	chr19: 44,905,796-44,909,393
PRNP	Prion D	chr20: 4,686,350-4,701,590
GRN	FTD	chr17: 44,345,246-44,353,106
C9orf72	FTD-ALS	chr9: 27,546,546-27,573,481
MAPT	FTD	chr17: 45,894,554-46,028,334
VCP	FTD-ALS	chr9: 35,056,064-35,072,627
TARDBP	FTD-ALS	chr1: 11,012,344-11,025,739
FUS	ALS	chr16: 31,180,139-31,191,605
SOD1	ALS	chr21: 31,659,666-31,668,931
LRRK2	PD	chr12: 40,196,744-40,369,285
PRKN	PD	chr6: 161,347,417-162,727,775
SNCA	PD	chr4: 89,724,099-89,837,161

Abbreviations

APP: Amyloid precursor protein, PSEN1: Presenilin 1, PSEN2: Presenilin 2, APOE: Apolipoprotein E, PRNP: Prion protein, GRN: Progranulin, C9orf72: Chromosome 9 open reading frame 72, MAPT: Microtubule-associated protein tau, VCP: Valosin-containing protein, TARDBP: Transactive response DNA binding protein, FUS: Fused In Sarcoma, SOD1: Superoxide dismutase 1, LRRK2: Leucine-rich repeat kinase 2, PRKN: Parkin, SNCA: Synuclein Alpha. AD: Alzheimer's disease, Prion D: *Prion* diseases, FTD: Frontotemporal dementia, FTD-ALS: Frontotemporal dementia and amyotrophic lateral sclerosis, ALS: Amyotrophic lateral sclerosis, PD: Parkinson's disease.

Table S2: Observerd variants in genes associated with AD (*APP, PSEN1, PSEN2, APOE*) and the FTD-ALS spectrum (*MAPT, TARDBP, GRN, FUS, SOD1, VCP*), and PD (*LRRK2, PRKN, SNCA*)

Missense variations

gene	region	genomic mutation	predicted protein	ref SNP ID	frequency	polyphen-2	SIFT	SNP&GO
PSEN1	exon9	c.953A>G	p.E318G	rs17125721	3/30	benign	tolerated	neutral
GRN	exon11	c.1297C>T	p.R433W	rs63750412	1/30	benign		
SOD1	exon 4	c.272A>C	p.D91A	rs80265967	1/30	benign	tolerated	neutral

Deletion

gene	region	genomic mutation	predicted protein	ref SNP ID	frequency
PRNP	exon2	c.204_227del	p.P84_Q91	rs1389957	1/30

Frameshift mutations

gene	region	genomic mutation	predicted protein	ref SNP ID	frequency
LRRK2	exon 34	c.4915del	p.R1639G fs*14	rs756089224	15/30
PRKN	exon11	c.1283del	p.N428Mfs*6		22/30

Silent variantions

gene	region	genomic mutation	predicted protein	ref SNP ID	frequency
PRNP	exon2	c.351A>G	p.A117A	RS8124214	1/30
PSEN2	exon4	c.69T>C	p.A23A	rs11405	26/30
	exon4	c.129C>T	p.N43N	rs6759	24/30
	exon5	c.261C>T	p.H87H	rs1046240	24/30
	exon6	c.414C>T	p.S138S	rs747738607	1/30
MAPT	exon9	c.1479G>A	p.P493P	rs1052551	8/30
	exon10	c.1632A>G	p.A544A	rs1052553	8/30
	exon10	c.1716T>C	p.N572N	rs17652121	8/30
	exon10	c.1761G>A	p.P587P	rs1568305	8/30
GRN	exon5	c.384T>C	p.D128D	rs25646	1/30
FUS	exon3	c.147C>A	p.G49G	rs781810	11/30
	exon4	c.291C>T	p.Y97Y	rs76570520	20/30
	exon12	c.1197T>G	p.G399G	rs76570520	5/30
LRRK2	exon10	c.1104G>A	p.E368E	rs1243256303	13/30

Observed variants in 30 pathology-confirmed CJD patients. For the rare variants with MAF ≤ 1%, in silico modeling of pathogenicity was performed using SIFT (http://sift.jcvi.org/), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/), and SNP&GO (https://snps-and-go.biocomp.unibo.it/snps-and-go/).

Table S3: Clinical presentation of sCJD cases.

Number (CMN)	Age of onset	Disease duration (month)	Age of death	Gender	Family history	Pathology	Biochemical subtype	PRNP (SNP 129)	Neuropathological diagnosis PART ARTAG AD	Ataxia	Disorientations	Sensitive disorders	Cognitive deficits	Walking instability	Extrapyramidal signs	Phatic disorders	Tremor	Visual symptoms	Myoclonus	Word disorders	Dementia	Behavioral disorders
FM8/18	71	1	71	F	S	PPR,AD	1	MM	sCJD, A2B1C2	x	x		x	x				x	x		x	
MA95/17	69	2	69	F	S	PPR, AD	1	MM	sCJD, A2B2C1		x			x						x	x	
ČV102/17	68	2	68	F	S	PPR, AD	1	MM	SCJD, A2B2C2		x		x	x						x	x	
ŠS1/18	70	2	70	F	S	PPR, AD	1	VV	sCJD, A2B2C2	x			x		x	x		x			x	
ŠP9/19	75	1	75	M	S	PPR, AD	1	MM	sCJD, A2B2C2		x		x			x					x	
EA65/19	79	1.5	79	M	S	PPR, AD	1	MM	sCJD, A2B2C2									x		x	x	
LF48/18	83	1	83	M	S	PPR, AD	1	MM	sCJD, A2B2C2		x	x	x		x				x		x	
KJ30/19	75	2	75	M	S	PPR, AD	1	MM	sCJD, A2B2C2	x	x		x	x			x	x	x		x	
PA135/16	64	1.5	64	M	S	PPR, AD	1	MV	sCJD, A2B2C2	x	x							x	x		x	
PJ30/18	62	6	62	M	S	PPR, AD	2A	MV	sCJD, A3B2C2										x		x	
BP64/17	63	2	63	M	S	PPR	1	MM	sCJD				x							x	x	x
ZD125/17	47	2	47	M	S	PPR	1	MV	sCJD		x								x	x	x	x
LT153/17	57	6	57	M	S	PPR	1	VV	sCJD				x	x	x				x		x	
BM10/8	75	2	75	F	S	PPR	1	VV	sCJD					x							x	
AM13/18	65	2	65	F	S	PPR	1	MM	sCJD				x	x	x	x			x	x	x	
ŠJ14/18	54	24	56	F	S	PPR	2A	MM	sCJD		x		x								x	
VJ47/18	78	2	78	M	S	PPR	1	MM	sCJD		x	x	x	x			x	x		x	x	
KE139/18	59	2	59	F	S	PPR	1	MM	sCJD		x		x				x	x	x	x	x	
KI33/19	55	12	56	F	S	PPR	2A	MM	sCJD		x		x								x	
LF44/19	48	4	48	M	S	PPR	1	VV	sCJD	x			x	x	x	x	x	x	x	x	x	
AF112/16	81	2	81	F	S	PPR, tau	1	VV	sCJD, PART	x	x	x	x								x	
ŠL113/16	72	4	72	M	S	PPR, tau	1	MM	sCJD, PART			x	x	x	x	x		x			x	
HB123/16	55	10	56	M	S	PPR, tau	2	MM	sCJD, PART	x			x	x	x		x	x			x	
FJ15/18	59	2	59	M	S	PPR, tau	1	MV	sCJD, PART		x		x	x				x		x	x	
HJ93/18	75	2	75	M	S	PPR, tau	1	MM	sCJD, PART							x			x		x	
VJ27/19	87	1-2	87	M	S	PPR, tau	1	MM	sCJD, PART	x				x				x	x		x	
KA90/17	74	9	75	F	S	PPR, AD	1	VV	sCJD, A1B1C1					x		x			x	x	x	x
MF20/18	68	10	69	M	S	PPR, AD	1	MV	sCJD, A1B1C1												x	
KE66/18	65	5	65	F	S	PPR, AD	1	VV	cCJD, A1B1C1	x	x	x		x					x		x	
SI56/19	65	6	65	F	S	PPR, AD	1	VV	sCJD, A1B2C1					x	x				x	x	x	

Symbols: S: sporadic, F: familial, PPR = prionopathy, AD: β-amyloidopathy, tau: tauopathy, M: male, F: female, PART: primary age-related tauopathy, ARTAG: aging-related tau astrogliopathy, MM: methionine/methionine, MV: methionine/valine, VV: valine/valine.

Table S4: Association between CJD and *PRNP* gene polymorphism at codon 129 defined by *APOE* ε4 allele status

sCJD genotypes

	ε2/ ε2	ε2/ ε3	ε2/ ε4	ε3/ ε3	ε3/ ε4	ε4/ ε4
M129M	0	2	0	2	2	0
M129V	0	0	0	1	0	0
V129V	0	0	0	2	1	0

sCJD and tauopathy and/or early evolved AD

	ε2/ ε2	ε2/ ε3	ε2/ ε4	ε3/ ε3	ε3/ ε4	ε4/ ε4
M129M	0	1	0	3	0	0
M129V	0	0	0	1	1	0
V129V	0	0	0	4	0	0

sCJD and developed AD

	ε2/ ε2	ε2/ ε3	ε2/ ε4	ε3/ ε3	ε3/ ε4	ε4/ ε4
M129M	0	0	0	4	1	2
M129V	0	1	0	0	0	1
V129V	0	0	0	1	0	0

Table S5: Genotype APOE and haplotype MAPT

dnumber (CMN)	APOE (genotype)	MAPT (haplotype)	PRNP	Neuroptahological diagnosis
FM8/18	ε3ε3	H1/H1	negative	sCJD,
MA95/17	ε3ε3	H1/H1	negative	sCJD,
ČV102/17	ε3ε4	H1/H1	negative	SCJD,
ŠS1/18	ε3ε3	H1/H2	negative	sCJD,
ŠP9/19	ε4ε4	H1/H1	p.P84_Q91	sCJD,
EA65/19	ε3ε3	H1/H1	negative	sCJD,
LF48/18	ε4ε4	H1/H1	negative	sCJD,
KJ30/19	ε3ε3	H1/H2	negative	sCJD,
PA135/16	ε2ε3	H1/H2	negative	sCJD,
PJ30/18	ε4ε4	H1/H2	negative	sCJD,
BP64/17	ε3ε3	H1/H1	negative	sCJD
ZD125/17	ε3ε3	H2/H2	negative	sCJD
LT153/17	ε3ε3	H1/H2	negative	sCJD
BM10/8	ε3ε4	H1/H1	negative	sCJD
AM13/18	ε3ε3	H1/H1	negative	sCJD
ŠJ14/18	ε2ε3	H1/H1	negative	sCJD
VJ47/18	ε2ε3	H1/H2	negative	sCJD
KE139/18	ε3ε4	H1/H2	negative	sCJD
KI33/19	ε3ε4	H1/H1	negative	sCJD
LF44/19	ε3ε3	H1/H1	negative	sCJD
AF112/16	ε3ε3	H1/H1	negative	sCJD,
ŠL113/16	ε2ε3	H1/H2	negative	sCJD,
HB123/16	ε3ε3	H1/H1	negative	sCJD,
FJ15/18	ε3ε3	H1/H1	negative	sCJD,
HJ93/18	ε3ε3	H1/H1	negative	sCJD,
VJ27/19	ε3ε3	H1/H1	negative	sCJD,
KA90/17	ε3ε3	H1/H1	negative	sCJD,
MF20/18	ε3ε4	H1/H1	negative	sCJD,
KE66/18	ε3ε3	H1/H2	negative	sCJD,
SI56/19	ε3ε3	H1/H2	negative	sCJD,

Symbols: sCJD sporadic Creutzfeldt-Jakob disease

Table S6: List of new genes

Disease Categories	Candidate Genes Selection
Alzheimer's disease	APP, PSEN1, PSEN2, S100A9, CR1, BIN1, TREM2, CLU, CTNNA3, DNMBP, SORL1, BACE1, PICALM, GAB2, LPR6, ADAM10, ABCA7, CD33, TOMM40
Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal dementia (FTD)	TDP43, CHMP2B, SIGMAR1, VCP, FUS, GRN, MAPT, UBQLN2, ALS2, TAF15, FIG4, OPTN, DAO, HNRNPA1, SOD1, ANG, VAPB, SQSTM
Dementia with Lewy Bodies	PINK1, PARK7, PARK9, GBA, SNCA, PRKN, LRRK2
Other neurodegenerative	SPAST, CYP7B1, SPG11, CSF1R, NOTCH3, PRNP

13.2. FENOTYPOVÉ A GENOTYPOVÉ POROVNÁNÍ VZÁCNÝCH PŘÍPADŮ GSS ZACHYCENÝCH V ČESKÉ REPUBLICE

Adam Tesar, MD, Radoslav Matej, MD, PhD, Jaromir Kukal, PhD, Silvie Johanidesova, MSc, Irena Rektorova MD, PhD, Martin Vyhnalek MD, PhD, Jiri Keller MD, PhD[1], Ilona Eliasova MD, PhD, Eva Parobkova MSc, Magdalena Smetakova MSc, Zuzana Musova, PhD, Robert Rusina MD, PhD. **Clinical Variability in P102L Gerstmann-Sträussler-Scheinker Syndrome.**

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Clinical Variability in P102L Gerstmann–Sträussler–Scheinker Syndrome

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Gerstmann–Sträussler–Scheinker syndrome (GSS) with the P102L mutation is a rare genetic prion disease caused by a pathogenic mutation at codon 102 in the prion protein gene. Cluster analysis encompassing data from 7 Czech patients and 87 published cases suggests the existence of 4 clinical phenotypes (typical GSS, GSS with areflexia and paresthesia, pure dementia GSS, and Creutzfeldt–Jakob disease–like GSS); GSS may be more common than previously estimated. In making a clinical diagnosis or progression estimates of GSS, magnetic resonance imaging and real-time quaking-induced conversion may be helpful, but the results should be evaluated with respect to the overall clinical context.

ANN NEUROL 2019;00:1–10

Gerstmann–Sträussler–Scheinker syndrome (GSS; Online Mendelian Inheritance in Man database 137440) is a very rare slowly progressive disease, caused by a mutation in the prion protein gene (*PRNP*), mostly a proline-to-leucine substitution at residue 102 (P102L), with autosomal dominant transmission (a significant fraction of previously reported cases were attributed to a sporadic mutation). P102L GSS is characterized by varying degrees of prion-protein–immunoreactive amyloid deposits, reactive astrogliosis, and in a majority of cases, spongiform encephalopathy in different brain regions, mainly in the cerebral and cerebellar cortices and the basal ganglia.[1]

The estimated prevalence is 1 to 10/100,000,000, with onset typically in the 5th decade (51.5 ± 12.8 years; although, in recent reports, it is 41 ± 14 years).[2–6] The average illness duration is 40 to 50 months (44 ± 12.1 months, median = 40).[2,4,5]

The most common clinical phenotype includes early ataxia with gait disturbances, sensory involvement in the lower extremities, and late cognitive decline,[4] whereas visual disturbances, dystonia, and myoclonus are uncommon.[7–9]

The clinical presentation, however, can be highly variable,[4] including cases with prominent early neuropathy (hyporeflexia, fasciculation, amyotrophy, and sensory loss),[10] patients with painful dysesthesias co-occurring with parkinsonism and lower leg spasticity, atypical cases mimicking progressive supranuclear palsy, and cases with optic atrophy.[9,11,12] GSS can also be clinically indistinguishable from sporadic Creutzfeldt–Jakob disease (CJD).[8]

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Additional supporting information can be found in the online version of this article.

The underlying cause of this remarkable variability remains poorly understood; however, suggested causes include codon 129 or the distribution of atypical forms of wild-type prion protein (PrP) in different brain areas.[4,13–15]

In this Neurology Grand Rounds article, we use a multifaceted approach to better characterize the variable clinical presentation seen in P102L GSS patients. The first step was to retrospectively analyze the clinical manifestation and results from auxiliary diagnostic methods (ie, brain magnetic resonance imaging [MRI], electroencephalogram [EEG], and cerebrospinal fluid [CSF] analysis) in the 7 Czech cases with postmortem confirmation. The next step was to extend this analysis by combining the data from the 7 Czech patients with data from 87 previously published P102L GSS cases,[7,8,10–14,16–42] and with the use of cluster analysis, to define typical GSS syndrome phenotypes.

Characteristics of Our Reported 7 Cases (Set A)

In the database of the National Reference Laboratory for Human Prion Diseases, Thomayer Hospital, Prague, we identified 7 P102L GSS cases in a 10-year period 2008–2018 (from a population of 8,749,172 adult inhabitants; Czech statistical office, https://www.czso.cz/csu/czso/age-distribution-of-the-population-2016) and denominated them as Set A (Table 1).

We performed our data analysis in accordance with the Declaration of Helsinki. The analysis was also approved by the Multicenter Ethics Committee of the Institute of Clinical and Experimental Medicine and Thomayer Hospital in Prague, Czech Republic (approval no. G1827). All analyzed patient medical data were anonymized. Clinical data are summarized in Supplementary Table 1.

Two and 2 patients, respectively, belonged to the same family, and all patients were native Czechs. From this information, we then estimate the prevalence of GSS as 8/10,000,000. Using a critical level of 0.05, we obtained a confidence interval (CI) for the posterior beta distribution as a prevalence of CI = 3 to 14 per 10,000,000, which is a relatively wide interval.

The median age of disease onset was 57 years (interquartile range [IQR] = 40.0–59.5), and the median disease duration was 27.6 months (IQR = 10.5–53.5), with a 4:3 male to female ratio. The *PRNP* polymorphism at codon 129 was identified in the entire dataset of Czech patients; all cases have methionine at codon 129 in the mutated allele.

We defined dementia duration as the period starting at the onset of cognitive deterioration impacting on activities of daily living and continuing until death. Dementia was present in 5 cases with a median onset of 58 years (IQR = 44–62) and a median duration of 3 months (IQR = 1–13.8). Deep tendon reflexes were abnormal in 3 patients (lower limb areflexia was present in 2 patients, and hyperreflexia was present in 1), painful lower limb dysesthesias were present in 2 patients, and paresthesia was present in 1.

EEGs were available in 5 cases; periodic triphasic patterns were seen in 2 cases, and epileptiform discharges were seen in 1 case. All our patients had an MRI with axial diffusion-weighted imaging (DWI) and fluid-attenuated

TABLE 1. Summary of Descriptive Data of Czech Patients with GSS since 1999

Case	Gender	Age at Disease Onset, yr	Duration of Disease, yr	Dementia Onset after Disease Onset, yr	Ataxia Onset after Disease Onset, yr	Changes in Reflexes[a]	Sensory Symptoms[b]	Leu 102 in Cis With	GSS Subtype
1	F	43	1.333	1.083	0	1	3	129 Met	Typical GSS
2	M	62	0.417	0.333	0.25	0	0	129 Met	CJD-GSS
3	M	65	0.333	0.333	X	0	0	129 Met	CJD-GSS
4	M	57	3.917	X	0	3	3	129 Met	Typical GSS
5	F	29	9	X	1	3	2	129 Met	Typical GSS
6	M	37	2.3	1.150	0.08	0	0	129 Met	Typical GSS
7	F	57	5	1.000	0	0	0	129 Met	GSS with areflexia and paresthesia

GSS subtypes include typical GSS, GSS with areflexia and paresthesia, pure dementia GSS, and CJD-GSS.
[a]Changes in reflexes: 0, no change; 1, hyperreflexia; 2, changes only in upper limbs; 3, areflexia in lower limbs.
[b]Sensory symptoms: 0, no symptoms; 1, anesthesia; 2, paresthesia; 3, dysesthesia.
F = female; M = male; X = undefined value; GSS = Gerstmann–Sträussler–Scheinker syndrome; CJD-GSS = Creutzfeldt–Jakob disease–like GSS.

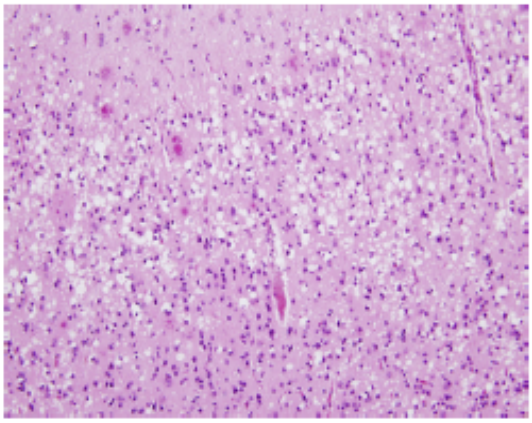

FIGURE 1: Prominent spongiform changes and multiple amyloid plaques in cerebral cortex seen in standard hematoxylin–eosin staining in Patient 5. Original magnification, ×200.

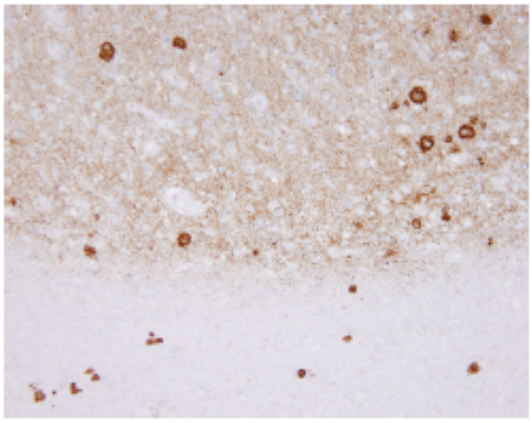

FIGURE 2: Prion protein (PrP) immunoreactivity in the cerebral cortex of Patient 7 shows a diffuse, synaptic staining seen in association with multiple PrP-immunopositive plaques in cortex and subcortical white matter. Immunolabeling with 12F10; original magnification, ×200.

inversion recovery (FLAIR) sequences; basal ganglia (mainly putaminal and caudate) hyperintensities evoking CJD in DWI and/or FLAIR sequences were seen in 5 subjects (71.4%), cerebellar atrophy was seen in 4 cases, and pronounced generalized corticosubcortical atrophy was seen in 1 case. Basic CSF analysis (cell count, protein, and glucose levels) was performed in all cases and was found to be normal. In 1 sample (from 4 tested), protein 14-3-3 was positive; in all tested samples, total tau levels were significantly increased (>1,200 ng/l, n = 200–450); phosphotau and amyloid beta peptides were slightly abnormal in 3 cases (see Supplementary Table 1).

Two patients fulfilled the modified World Health Organization diagnostic criteria for probable sporadic CJD, and 2 patients had clinical presentations similar to the initial GSS description published in 1936.[43]

During autopsies, spongiform dystrophy of varying severity was observed in cortical and subcortical areas (Fig 1) in all cases. We also noted different degrees of neuronal atrophy and reactive astrogliosis. Neuropathological hallmarks of GSS with prominent amyloid plaques, which tested positive in immunohistochemical reactions with anti-PrP antibodies (Fig 2), were found in both the cerebellar and cerebral cortex, and in the subcortical gray and white matter structures.

Characteristics of Our Reported 7 Cases Compared to Previously Published Data

We analyzed all case reports of GSS with a genetically or genealogically demonstrated P102L mutation available in the literature since 1992, using PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), Scopus (https://www.scopus.com/home.uri), and the Web of Science Core Collection databases (https://apps.webofknowledge.com/).

We excluded 2 articles written in Japanese to prevent translation-related misinterpretations, and we lacked complete access to a third article.[9,44,45]

We focused on gender, age at onset, disease duration, onset and duration of dementia (from the onset of cognitive deterioration impacting on activities of daily living and continuing until death), onset of ataxia, MRI abnormalities (in particular basal ganglia, cortex, and cerebellum), polymorphism in codon 129, changes in deep tendon reflexes and sensory symptoms, and 14-3-3 protein in the CSF.[7,8,10,11,13,14,16–42]

Single or Small Case Series (Set B)

This dataset (Set B) included data both from our 7 Czech patients and from published single cases and/or small case series; of 94 patients (Supplementary Table 2), 64 had died, and 30 were alive at the time of case publication; in 1 case, information about illness duration was unavailable.[29]

The median age of disease onset was 49 years (IQR = 37.25–57.83), and median disease duration was 48 months (IQR = 36–72) in the deceased patients and 48 months (IQR = 24–72) in all (deceased and living) patients. Disease duration was not significantly influenced by the inclusion or exclusion of patients still alive at publication (ie, unknown illness duration until death) with $p = 0.4274$. The male:female ratio was 0.78 (38:49); gender was not specified in 7 patients.

Dementia was diagnosed in 63 patients (67.02%; median onset at 50.83 years, IQR = 38.08–58.75; median duration = 24 months, IQR = 12–46.5) and ataxia in 84 subjects (89.36%; median onset during disease at 2 months, IQR = 0–12), and 10 patients (10.64%) manifested dominant psychiatric symptoms (paranoia or schizophrenia in 3, auditory

or visual hallucinations in 4, and behavioral changes in 3 patients).

Abnormal deep tendon reflexes were found in 44 subjects (46.81%; lower limb areflexia in 25 cases, 26.60%; hyperreflexia in 10 cases, 10.64%). Sensory complaints in the lower extremities occurred in 32 cases (34.04%; dysesthesias in 20 individuals, 21.28%; paresthesia in 5 cases, 5.32%; hypesthesia in 7 cases, 7.45%).

Dysesthesias were common in patients with lower limb areflexia ($p = 6.1343 \times 10^{-9}$) and linked to later disease onset (59 vs 47 years, $p = 0.0036$), which agrees with results from the cluster analysis (see below).

EEGs were done in 57 subjects; only 5 patients (8.77%) had findings suggesting prion disease (triphasic generalized complexes in 3 and periodic synchronic activity in 2 cases).

Neuroimaging was available in 69 patients; 4 had computed tomography (CT; 2 showed cortical atrophy, 1 had atrophy of the cerebellum, and 1 CT was normal) and 65 had MRIs with abnormal findings (Fig 3) found in 51 patients (78.46%; CT/MRI cortical atrophy in 38 patients, 55.07%; MRI cortical hyperintensities in 21 subjects, 32.32%; MRI basal ganglia hyperintensities in 14 patients, 21.54%; CT/MRI cerebellar atrophy in 29 patients, 42.03%). All our patients had an MRI with axial DWI and FLAIR sequences; basal ganglia (mainly putaminal and caudate) hyperintensities evoking CJD in DWI and/or FLAIR sequences were seen in 5 subjects (71.4%). Basal ganglia hyperintensities were found to be related to shorter disease duration (median = 24.5 vs 48 months, $p = 0.0329$).

CSF tau and amyloid beta testing was reported in only 31 patients; 7 patients had high total tau levels, 1 had elevated amyloid beta, 3 had hyperproteinorhachia, and in 17 patients, the CSF analysis was normal. Protein 14-3-3 was positive in 6 of 12 tested patients (50%). Real-time quaking-induced conversion (RT-QuIC) test results for GSS were only reported in a few articles and appeared to have a sensitivity up to 75 to 78%.[46,47]

We did not identify any clinical differences between sporadic, possible familial, or familial cases (see Supplementary Table 2); the sporadic variant had a slightly later disease onset (53 vs 47 years) and slightly longer disease duration (5 vs 4 years), but without statistical significance ($p = 0.28$ and $p = 0.68$, respectively).

We noticed considerable variability between members of the same family. We focused on testing this variability by comparing differences in onset and disease duration between random pairs (using a random number generator), between child–parent pairs, and between 2 siblings. Differences in the median age at onset between siblings were smaller than in random pairs (median = 7 vs 13 years, $p = 0.0017$), but nonsignificant between child–parent pairs and random pairs (median 9 years, $p = 0.1061$) or between siblings and child–parent pairs ($p = 0.2005$; Fig 4). Disease duration and phenotypical presentation (dementia, ataxia, paresthesia, psychiatric features, and insomnia) did not significantly differ between different pairings (random couples vs pairs of parents and children, vs siblings).

We also focused on polymorphism 129 of the *PRNP* gene in GSS. In this dataset (Set B), in 18 cases, there unfortunately were no data about polymorphism 129; however, in

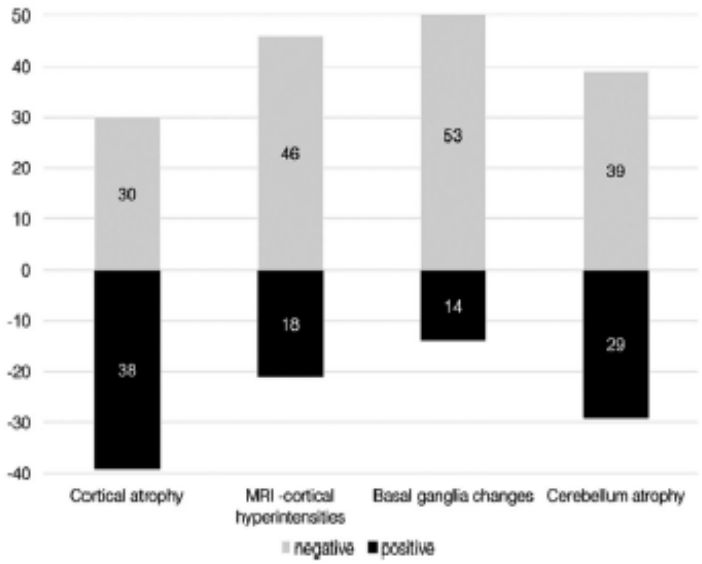

FIGURE 3: Results of neuroimaging (magnetic resonance imaging [MRI]/computed tomography) methods in our patients and published cases.

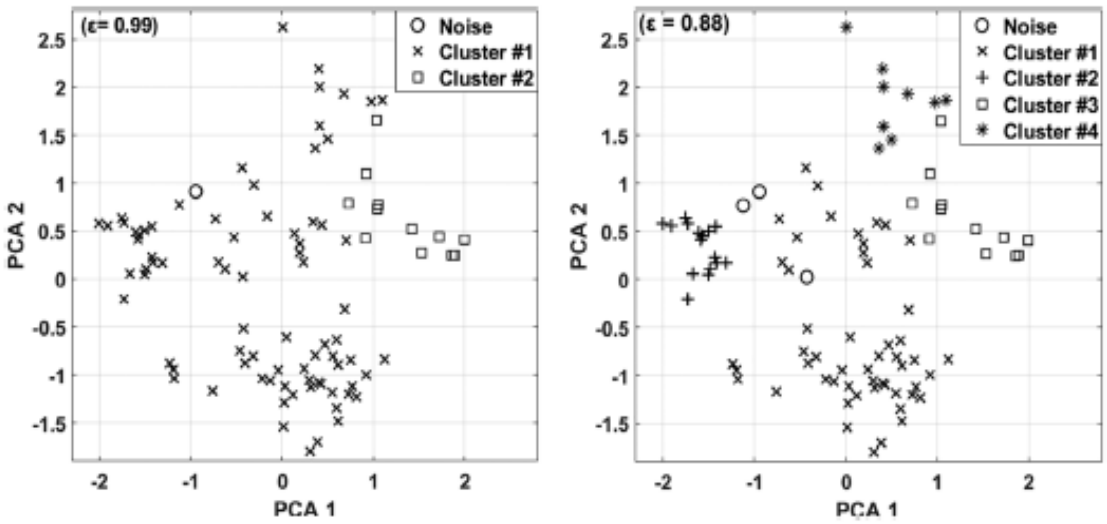

FIGURE 4: Results of cluster analysis of 93 patients with verified P102L Gerstmann–Sträussler–Scheinker syndrome showing clusters of patients with different phenotypical presentations. Horizontal axis: values of the first principal component analysis (PCA 1); vertical axis values: values of PCA 2.

70 patients, codon 129 of the mutated allele coded for methionine (identified genetically or genealogically); in 6 patients, valine was coded for by codon 129. The presence of methionine was linked to later disease onset (median = 52.5 vs 38 years, $p = 0.0055$) and was associated with shorter disease duration (median = 42 vs 51.6 months, $p = 0.0318$).

All Published Cases (Set C)

This dataset matched previous data (Set B) with data from 46 patients with P102L GSS from a large multicenter survey[4] and 5 patients from a study by Parchi et al[48]; however, because of missing data about dementia onset and ataxia in Parchi's cohort, we excluded these patients from our cluster analysis.[4,48]

The total number of patients in Set C was 145, with a median age at onset of 49 years (IQR = 39–57) and a median disease duration of 48 months (IQR = 36–72; this was the same when living patients were included); the male:female ratio was 0.68 (56:82).

Dementia was found in 100 patients (68.97%) and ataxia in 129 patients (88.97%), 67 patients had abnormal deep tendon reflexes (45.89%), and 51 patients had sensory symptoms (35.17%).

Using the Wilcoxon–Mann–Whitney test, we found that patients with abnormal lower limb reflexes were more likely also to have sensory symptoms ($p = 3.4132 \times 10^{-11}$); patients with ataxia had a later onset of disease (median = 51 vs 38 years, $p = 0.0078$). Disease duration was longer and with an earlier onset for valine/valine 129 polymorphism (median duration = 11.5 years, $p = 0.0224$) with a median onset of 30 years ($p = 0.0430$; however, this polymorphism was only seen in 2 cases, and neither suffered from dementia). The allelic position of polymorphisms in codon 129 was not identified in any of the cases from the Webb et al and Parchi et al cohorts [4,48]

Toward Defining Typical Clinical Phenotypes of P102L GSS

Statistical Analysis

Data from all patients were analyzed using MATLAB software R2018a (MathWorks, Natick, MA). Mardia multivariate analysis of normality[49] testing was done to verify normal distributions of the dataset; p-skew and p-Kurt were both <0.001. Therefore, the dataset did not have a normal distribution, and descriptive statistics were compared with the nonparametric Wilcoxon–Mann–Whitney test at the $p = 0.05$ level of significance.[50]

Finally, a principal component analysis (PCA) and density-based sequential cluster analysis (DBSCAN) were used to define phenotypes.[51,52] Results of PCA showed that the entire system was described by 14 dimensions (the first 3 contained 62.74% of the variance, and the first 7 contained 90.19% of the variance). The advantages of DBSCAN are its reproducibility, focusing on dense regions referred to as cluster kernels, its ability to detect outliers, and the absence of prior cluster number declaration.

The DBSCAN is driven by 2 parameters. The minimal point number in a single cluster; we set this to 3 to ensure visibility of compact but small clusters in sets. The second parameter was the epsilon value, which specifies the critical distance between 2 points within a single cluster. We set the epsilon to reduce the number of outliers to

<5% (ie, a maximum of 4 patients as outliers) to reduce the number of clusters if low epsilon values were used.

We considered that dementia and ataxia onset could be crucial for the definition of phenotypes. Variable quality of available data from published articles does not enable establishing a possible range for onset (for instance, ataxia was mentioned only during follow-up examinations 1 year after the initial assessment). In such cases, we defined ataxia onset as the arithmetic average of these 2 values (ie, a half year in the cited example). In patients without manifest dementia or ataxia, the time from onset of dementia/ataxia was equal to disease duration (ie, in a patient with 3-year duration of dementia but no ataxia, we stated that the onset of ataxia was after 3 years).

To compare MRI findings, we defined 4 parameters: brain atrophy, abnormal signals in brain tissue, cerebellar atrophy, and basal ganglia hyperintensities. In most cases, only the terms "pathological" or "normal results" were available; thus, we used simplified categories (ie, present, absent, not tested).

Results of the DBSCAN Cluster Analysis

We analyzed data from 94 published cases (including our 7 Czech patients); 1 patient was excluded because of missing information about disease duration.[29]

First, we defined the number of dimensions from PCA. More dimensions mean better preservation of information; however, they also result in small clusters with minimal variety. Fewer dimensions lead to a simplification and greater variety between larger clusters. Therefore, we applied a gradual reduction in number of dimensions to clarify the most robust clusters to conserve the greatest possible information; 3 dimensions covered approximately 63% of all analyzed information.

Second, we progressively changed the epsilon parameter. For epsilon = 0.99, the dataset was divided into 2 clusters: (1) 79 patients with earlier disease onset (median 48 vs 56 years, $p = 0.0345$), a longer disease duration (median = 48 vs 7 months, $p = 1.5481 \times 10^{-6}$), and late onset dementia (median = 30 months, $p = 1.6093 \times 10^{-7}$); and (2) 13 patients with dominant dementia (median onset = 0).

Patients with neuropsychiatric features were included in both clusters alike (6 in the first and 4 patients in the second). Within the second cluster, only 1 patient had sensory symptoms, 3 patients had hyperreflexia, and 1 patient was an outlier.

After splitting the set, we tried to define subtypes of these 2 phenotypes; reducing the epsilon to 0.95 caused splitting of the first cluster (non–dementia dominant) into 2 subclusters. The first had 69 patients (median onset = 49 years), and the second had 9 patients (median onset = 35 years, $p = 0.0263$). Disease duration was the same for both clusters (median = 48 months), but ataxia onset was 0 months for the first and 36 months for the second cluster

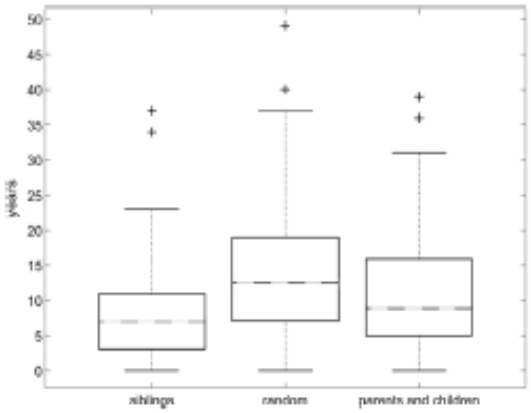

FIGURE 5: Intergenerational variability between pairs of siblings, between parents and children, and between random pairings.

($p = 1.0854 \times 10^{-6}$); dementia onset was delayed in the first cluster (36 vs 0 months, $p = 1.1147 \times 10^{-6}$).

By restricting epsilon to 0.88, the original first cluster was subdivided into 2 subclusters, with 16 and 52 patients, respectively. Patients in the smaller cluster had dominant areflexia ($p = 6.4346 \times 10^{-10}$), painful paresthesia/dysesthesias ($p = 8.6535 \times 10^{-12}$), and later onset of both ataxia (median onset = 12 months, $p = 0.0031$) and dementia (median onset = 30 months, $p = 0.2414$). Patients in the larger cluster had the same characteristics as patients in the original cluster of 69 patients. If we decrease epsilon, the clusters will be divided into higher number of small clusters and also the number of outliers (noise) will increase. But we defined before the beginning of the analysis a threshold for outliers as lower than 5% of set members. Otherwise we can defined any similarity between several patients as specific phenotype. Results are summarized in Figure 5 and Table 2.

Clinical Phenotypes of P102L GSS

Let us now apply this statistical framework to all available data to identify relevant GSS P102L clinical subtypes.

The first major phenotype group, mostly corresponding to the initial description by Gerstman, Sträussler, and Scheinker, consisted of 79 patients (84.04%) with late dementia (>36 months), early ataxia (median = 0 months), and longer disease duration (median = 48 months).[43] This group could be split into 3 subtypes: (1) typical GSS in 52 patients; (2) GSS with areflexia and paresthesia in 16 patients, with later onset of ataxia (median > 12 months) and later dementia (median > 30 months) than in the 2 other clusters; and (3) pure dementia GSS in 9 patients with early disease onset (median = 35 years, $p = 0.0241$), dominant dementia (median onset = 0 months), late ataxia onset (median = 36 months), and slow disease progression (median = 48 months).

TABLE 2. Summary of the Descriptive Characteristics of Different Clusters from Figure 3 (Epsilon = 0.85)

Cluster	Patients, n	Disease Onset, yr	Disease Duration, mo	Ataxia Onset, mo	Dementia Onset, mo	Sensory	Reflexes
1. Typical GSS	52	48	48	0	36	X	X
2. GSS with areflexia and paresthesia	16	57.5	36	12	30	Paresthesia	Areflexia
3. Creutzfeldt–Jakob disease–like GSS	13	56	7	0	0	X	X
4. Pure dementia GSS	9	35	48	36	0	X	X

GSS = Gerstmann–Sträussler–Scheinker syndrome.

The second major phenotype with dominant dementia, CJD-like GSS, was characterized by late onset (median onset = 56 years) with early dementia and ataxia and very rapid disease progression (median duration = 7 months, $p = 1.4275 \times 10^{-6}$). This subtype included all 3 cases previously reported to have this phenotype.[8] We did not identify a higher prevalence of neuropsychiatric features (hallucinations or paranoia) compared to typical GSS, and there was only 1 patient with sensory symptoms in this group.

Discussion

The main findings of our study are: (1) cluster analysis of data from our patients and previously published cases suggests 4 distinct GSS clinical phenotypes; (2) the difference in time of disease onset was significantly less between siblings than between random couples; (3) GSS may be more common than previously estimated; and (4) in making a clinical diagnosis or progression estimates of GSS, MRI and RT-QuIC may be helpful, but the results should be evaluated with respect to the overall clinical context.

First, cluster analysis identified 2 major phenotypical groups. The analysis showed similar results when applied to a random, but smaller, subset. Because uncertainty, in some cases, about the onset of ataxia or dementia (and ongoing disease in patients still alive) could impact data processing, we used different quantification methods (ie, an 8-point scale for different phenotypes related to dementia and ataxia onset before and after the median value, or differences in the duration of ataxia/dementia). All these approaches were concordant with results from cluster analyses (= same phenotypes), although with less accuracy.

Second, we focused on intergenerational variability. Within the same family, there was greater variability in manifestation, disease onset, and duration between 2 generations (parents and children). We found, however, that the age of disease onset was significantly more closely related in siblings compared to random pairings.

Wadsworth at al suggested that the spread of protease-resistant isoforms of wild-type PrP may significantly contribute to the phenotypic variability of P102L GSS.[15] This prion propagation, initially triggered by a *PRNP* mutation, principally involves PrP expressed from the wild-type allele.[15]

Affected siblings have a 50% probability of sharing the same wild-type allele and mutated allele, and this may cause similarities in the age of disease onset, whereas their parents and their children would only share the same mutated allele. Whichever is the case, further exploration is needed.

Methionine/methionine at codon 129 has been linked to earlier disease onset.[4] We observed, however, an inverse relationship in Sets B and C; we hypothesize that the onset of disease could result from a combination of wild-type and mutated alleles (which is true for different codon 129 polymorphisms). Codon 129 polymorphism was shown to be crucial to the clinical phenotype in CJD,[53] but it had no impact on phenotype presentation in GSS; however, we must note the very low number of published cases in which codon 129 of the mutated allele codes for valine.

Third, the currently estimated prevalence of GSS is 1 to 10/100,000,000.[3] Our data analysis, however, suggests a more common occurrence of GSS in the general population, which may be as high as 8 per 10,000,000. This estimation is difficult to apply directly to all published cases, because of (1) the long period of data acquisition (some patients died in the 1950s, others in 2017), (2) a retrospective diagnosis in many cases, and (3) inconsistencies in prion disease surveillance in different countries. There is, however, an important argument for our observation, which is the increase in new cases reported since 2017; we found a total of 26 patients, including 6 of our 7 Czech patients (1 of our cases was published and is therefore already counted in the literature).[27–29,34,36,37,41,42]

Fourth, we focused on neuroimaging, EEG, and CSF (14-3-3 assay and prion RT-QuIC) findings. In contrast to sporadic CJD, there are large discrepancies, and

heterogeneous findings in GSS[2,4,5,7] and intensity changes on MRIs can change significantly over the course of the disease.[23] On the other hand, both MRI (signal hyperintensity in the cerebral cortex or basal ganglia) and CSF analysis (14-3-3 assay and prion RT-QuIC) are positive in a significant number of cases. RT-QuIC, a new diagnostic tool that is highly specific for CJD, is considerably less sensitive for GSS, reaching 75 to 78% (however, there are only a limited number of tested cases).[46,47] In conclusion, for making a clinical diagnosis or progression estimates of GSS, MRI and RT-QuIC can be helpful, but the results should be (compared to diagnostic workup in sporadic CJD) evaluated with respect to the overall clinical context of the given patient.

In our set of 7 Czech patients (Set A; all 7 had a brain MRI), we evaluated cortical atrophy, cortical hyperintensities, cerebellar atrophy, and basal ganglia hyperintensities, and extended our findings to available data from published cases. The only significant finding was that basal ganglia hyperintensities correspond to shorter disease duration (24.5 vs 48 months, $p = 0.0329$). In 2 cases of genetically verified GSS P102L, voxel-based specific regional analysis system for Alzheimer disease detected thalamic atrophy (in line with decreased blood flow in the thalamus based on z score analysis using 99mTc-ethyl cysteinate dimer single photon emission computed tomography).[37] Unfortunately, thalamic voxel-based volumetry was not realizable in the MRI scans from our patients.

We also focused on EEGs, because typical triphasic complexes in 2 Czech patients were useful in confirming the clinical diagnosis of a prion disorder. However, EEGs from Set B were too diverse to be conclusive. Surprisingly, CSF analysis was not performed in the majority of cases (or the type of measurement was not specified). Therefore, its potential usefulness in GSS patients remains difficult to assess.

Our study is primarily a clinical study based on an analysis of our clinical data and a comparison to data from previously published case reports and case series. A major limitation of our study is that it was not possible to analyze pathological samples from the entire dataset. Therefore, an explanation of the underlying pathological mechanisms, beyond the phenotypical variability of P102 GSS, will require further neuropathological research.

So far, the reason for phenotypical heterogeneity in GSS is still unclear. No correlation of clinical signs with the localization of deposits of mutated *PRNP* has been documented, but it could be related to deposits of wild-type *PNRP* or to other *PRNP*.[15] We hypothesize that the 3 discussed phenotypes (typical GSS, GSS with areflexia and paresthesia, GSS with dominant dementia) could be caused by different involvement of central nervous system structures. Therefore, the later onset in the GSS with areflexia and paresthesia phenotype could be explained by the very slow spreading of the disease from the spinal cord to the brain, but we were not able to recognize any neuroimaging marker or differences in polymorphism 129.

An important methodological issue is the relevance and homogeneity of our datasets. In data provided by case reports (searched via the PubMed and Scopus databases) for all patients with the P102L mutation (verified genetically or genealogically), the median disease onset was slightly lower but nonsignificantly; the median of disease duration corresponded to published data.[2,4,6] We observed a slightly higher proportion of males compared to the literature.[1,6] We would expect a higher proportion of female patients based on the aging patterns of the general population, that is, there is a higher proportion of older women in developed countries, but this slight male predominance that we observed was in accordance with data in Webb's study, where most of the missing manifestations were males.[4]

Conclusion

GSS is a rare genetic prion disease that is probably more common than previously estimated, at least in the Czech Republic, and despite its clinical variability, a cluster analysis of our patients and data from previously published cases suggests that 4 different phenotypical groups can be identified (typical GSS, GSS with areflexia and paresthesia, CJD-like GSS, and pure dementia GSS), each with a distinct disease duration and a distinct set of clinical manifestations.

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Author Contributions

A.T., R.M., I.R., M.V., J.Ku, and R.R. contributed to the conception and design of the study; all authors contributed to the acquisition and analysis of data; A.T., R.M., and R.R. contributed to the drafting of the text and preparation of figures and tables; all authors contributed to the critical revision of the manuscript.

Potential Conflicts of Interest

Nothing to report.

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13.3. KOMPLEXNÍ ANALÝZA GENŮ ASOCIOVANÝCH S ALS/FTLD - PODOBNOSTI V GENETICKÉM POZADÍ.

Eva Parobkova, Radoslav Matej.

Amyotrophic lateral sclerosis and frontotemporal lobar degenerations: Similarities in genetic background.

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Review

Amyotrophic Lateral Sclerosis and Frontotemporal Lobar Degenerations: Similarities in Genetic Background

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Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating, uniformly lethal progressive degenerative disorder of motor neurons that overlaps with frontotemporal lobar degeneration (FTLD) clinically, morphologically, and genetically. Although many distinct mutations in various genes are known to cause amyotrophic lateral sclerosis, it remains poorly understood how they selectively impact motor neuron biology and whether they converge on common pathways to cause neuronal degeneration. Many of the gene mutations are in proteins that share similar functions. They can be grouped into those associated with cell axon dynamics and those associated with cellular phagocytic machinery, namely protein aggregation and metabolism, apoptosis, and intracellular nucleic acid transport. Analysis of pathways implicated by mutant ALS genes has provided new insights into the pathogenesis of both familial forms of ALS (fALS) and sporadic forms (sALS), although, regrettably, this has not yet yielded definitive treatments. Many genes play an important role, with *TARDBP*, *SQSTM1*, *VCP*, *FUS*, *TBK1*, *CHCHD10*, and most importantly, *C9orf72* being critical genetic players in these neurological disorders. In this mini-review, we will focus on the molecular mechanisms of these two diseases.

Keywords: amyotrophic lateral sclerosis; frontotemporal dementia; genetics; neuropathology

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating, uniformly lethal progressive degenerative disorder of motor neurons that overlaps with frontotemporal lobar degeneration (FTLD) [1]. ALS is a motor neuron disorder (MND), lateral sclerosis, and spinal muscular atrophies [2]. ALS has a worldwide incidence of about two per 100,000 per year, and a prevalence of about five per 100,000 [3]. Clinical symptoms include weakness of the bulbar and limb muscles, hyperreflexia, spasticity of the arms and legs, and respiratory failure. These symptoms most commonly develop between 40 and 70 years of age, although a wider age range has been reported [4]. Although most cases of ALS are sporadic (sALS), ≈10% are familial (fALS), with predominantly autosomal dominant transmission [5]. Sporadic ALS accounts for most ALS cases, although genetic causes are also known to play a role [6]. fALS is linked to mutations at a specific genetic locus [7]. The clinical and pathological presentation of fALS and sALS are similar [8]. Many sALS studies (GWAS) have identified genes associated with ALS disease [9]. ALS is closely related to frontotemporal lobar degeneration (FTLD) [10]. Many studies have shown clinical, pathological, and genetic commonalities between them. Therefore, they are now considered two manifestations of one disease continuum, i.e., the ALS-FTD spectrum or the FTD-ALS spectrum with the broader name, i.e., frontotemporal lobar degeneration, being associated with motor neuron disorders (FTLD-MND) [11]. FTD-ALS patients have a poor prognosis with a mean

survival of 2–3 years from the first onset of symptoms [12–14]. The reported heritability of FTLD-ALS is high: Approximately 50% of cases are considered familial [15,16]. ALS shares a common molecular etiology with frontotemporal dementia (FTD) [17], and approximately 15% of FTD patients develop motor neuron disease (MND), and conversely, up to 50% of MND show either direct signs of cognitive impairment [18,19] or minimal-mild disturbances in executive function [20,21]. These diseases are the extremes on a spectrum of clinically, pathologically, and genetically overlapping disorders [22], which suggests an overlap of disease mechanisms [23].

2. Mechanism and Molecular Pathology of ALS and FTLD

FTLD and ALS are two related neurodegenerative diseases forming the two ends of a disease spectrum. Behavioral FTLD is a form of dementia clinically characterized by progressive changes in behavior, personality, and language skills [24]. ALS involves the premature loss of upper and lower motor neurons, and a numeric decline in these neurons in the spinal cord, brainstem, and motor cortex [25], which leads to muscle weakness and atrophy [26]. The large number of genes and cellular processes associated with ALS has led to the suggestion that many disease mechanisms are involved. These include disturbances in RNA metabolism, impaired protein homeostasis, nucleocytoplasmic transport defects, impaired DNA repair, excitotoxicity, mitochondrial dysfunction, oxidative stress, axonal transport disruption, neuroinflammation, oligodendrocyte dysfunction, and vesicular transport defects [27]. While, traditionally, FTLD and ALS were considered to be two separate disease identities, it is now thought that FTLD and ALS form one clinical continuum, in which pure forms are linked by overlapping syndromes called FTLD-MND [17]. Recent advances in neuropathology and molecular genetics have started to reveal the biological basis for this clinical overlap. However, recent neuropathological findings suggest that FTLD cases present with distinct TDP-43 pathologies compared with ALS and FTLD-MND, indicating divergent disease pathogenesis mechanisms that nonetheless involve the same TDP-43 protein [28]. Although the underlying TDP-43 pathology does not always correlate with the genetics or disease phenotype, mutations of the progranulin (*GRN*) gene are generally associated with type A TDP-43 pathology, whereas hexanucleotide repeat expansions in *C9orf72*, which is the most common genetic cause of ALS and FTLD-TDP, most frequently result in the type B pathology [29,30]. Although *TARDBP* (the gene coding **protein TDP-43) mutations are rare in ALS and FTD (<1%), the pathological aggregation of** TAR DNA-binding protein 43 in affected brain regions and motor neurons is characteristic of the majority of ALS and FTD patients [11,31].

2.1. Protein and RNA Aggregates

The presence of protein and RNA aggregates in the cytoplasm of motor neurons is the hallmark of ALS [32]. The histopathological characteristics of ALS and FTD include abnormal accumulation of dysfunctional protein aggregations in the affected parts of the nervous systems [17]. Inclusions in ALS and FTD suggest that they share common pathogenic mechanisms leading to neurodegeneration and aggregation of specific inclusion proteins [33]. Similar ubiquitin-positive inclusions were observed in degenerating motor neurons of ALS patients [34], and aggregations of FUS protein have been reported in rare cases of ALS [35,36]. The most common protein inclusion in ALS is TDP-43 (encoded by the *TARDBP* gene) [32]; however, TDP-43 deposits have been observed in other neurodegenerative diseases such as Alzheimer's disease (A.D.) [37,38], Lewy bodies dementia (DLB) [39,40], and corticobasal degeneration (CBD) [38,41]. The relevance of the concomitant TDP-43 pathology remains unclear, and attempts to correlate a concurrent TDP-43 pathology with clinical phenotypes have provided mixed results [38,42]. *C9orf72* (chromosome 9 open reading frame 72) has an essential role in stress granule formation, microglial function, and autophagy [43–45]. The accumulation of (GGGGCC) might lead to sequestration of RNA binding proteins and disruption of the translation of diverse mRNA or increased nucleolar stress [46]. These abnormal protein aggregates are thought to be

the mechanism by which *C9orf72* expanded repeats contribute to ALS. One of the most frequently found proteins in neuropathological lesions is ubiquitin-binding protein p62 (sequestosome 1). Many experimental and clinical studies have shown that p62 plays a significant role in autophagy, an evolutionarily conserved pathway for the degradation of long-lived proteins and organelles. Dysfunction of the autophagy pathway may contribute to the pathology of various neurodegenerative disorders characterized by abnormal protein accumulation [47,48]. Recently, p62 was also shown to deliver ubiquitinated proteins, such as tau, and other crucial proteins involved in neurodegeneration, to proteasomes for degradation [49]. The build-up of p62-positive inclusions suggests defects in protein clearance pathways. Finally, a new role for p62 in maintaining mitochondrial integrity has recently been described [50]. A portion of p62 directly localizes within the mitochondria and supports stable electron transport by forming heterogeneous protein complexes [51]. Mutations in Superoxide Dismutase 1 (*SOD1*) produce an unstable protein deposited in the cytoplasm; oligomerization of unstable *SOD1* leads to aggregate formation [52]. Fused in sarcoma (*FUS*) is another RNA binding protein in which mutations can result in cytoplasmic aggregates. It is also a component of stress granules and may form p62 and TDP-43 positive aggregates [53]. It has been postulated that impaired autophagy could contribute to the accumulation of cytoplasmic aggregates [54]. Mutations in several autophagy genes have been associated with ALS, including Sequestosome 1 (*SQSTM1*), *SOD1*, optineurin (*OPTN*), valosin-containing protein (*VCP*), ubiquitin-2 (*UBQLN2*), and TANK-binding kinase 1 (*TBK1*) [55]. The removal of misfolded or damaged protein is critical for optimal cell functioning. In both the cytosol and the nucleus, major proteolytic pathways exist to recycle misfolded or damaged proteins, i.e., the UPS (ubiquitin proteasome system) and endosomal-lysosomal system (ELS) [56]. An impaired UPS is thought to be associated with the formation of proteinaceous inclusions in many neurodegenerative disorders [57].

2.2. Mitochondrial Dysfunction

Mitochondria dysfunction is at least partly responsible for the broad clinical spectrum of ALS and FTD. Mitochondrial dysfunction has been implicated in ALS motor neuron death [58]. Fragmentation of mitochondria and changes in mitochondrial morphology and expression of fusion/fission proteins are well described in ALS and have pronounced effects on normal mitochondrial function [59]. Mitochondria from ALS patients have impaired Ca^{2+} homeostasis and increased production of reactive oxygen species (ROS). ROS are associated with oxidative-related damage, including changes in protein carbonylates and tyrosine nitration [60]. Mitochondria are essential for cellular respiration, calcium buffering, and apoptosis. Neurons are particularly sensitive to mitochondrial dysfunction given their high metabolic rate [61]; as such, the presence of abnormal or dysfunctional mitochondria in neurons is thought to be a contributing factor in ALS. Mitochondria are of particular importance in neurons. Neurons have high metabolic requirements, with the brain consuming 20% of the body's resting ATP production despite being only 2% of the body's mass [62,63]. Moreover, mitochondria are essential calcium buffering organelles in neurons and function to modulate local calcium dynamics, for example, modulating neurotransmitter release [64]. Many proteins that have been linked to familial and sporadic ALS, including *SOD1*, *TDP-43*, *FUS*, and *C9orf72*, show interactions with mitochondria [65–67]. Direct evidence that disruption of mitochondrial structure (and as a result disruption of mitochondrial function; see below) may contribute to the etiology of ALS comes from the discovery of causative mutations in the mitochondrial protein Coiled-coil-helix-coiled-coil-helix domain-containing protein 10 (CHCHD10); this protein is localized to contact sites between the inner and outer mitochondrial membrane [68]. ALS-associated mutations in *CHCHD10* can disrupt mitochondrial cristae and profoundly affect the mitochondrial structure [69]. Mitochondrial DNA instability disorders are responsible for frontotemporal dementia [68]. In recent years, a growing list of FTD genes responsible for mitochondrial DNA instability has been reported [70]. The c.176C>T mutation in the *CHCHD10* gene was described in an FTD-ALS patient whose family was originally from

Catalonia (Spain), with affected individuals carrying a missense mutation in the *CHCHD10* gene. Functional characterization of the *CHCHD10* mutant identified in the family showed fragmentation of the mitochondrial network and the loss of cristae junctions [68]. *CHCHD10* is a novel gene responsible for the clinical spectrum of ALS-FTD, which raises the intriguing prospect of an underlying mitochondrial basis for this group of disorders.

2.3. Impaired DNA Repair

Impair DNA repair is another suggested mechanism that may contribute to ALS pathogenesis. Two of the best-studied ALS-linked proteins, TDP-43 and FUS, function to prevent or repair transcription-associated DNA damage [71]. FUS, in particular, seems to play an essential role in this regard and is involved in the repair of double-stranded DNA breaks via both homologous recombination and non-homologous end-joining repair mechanisms [72,73]. Variations in the genes of other ALS-linked RNA-binding proteins, including TATA-box binding protein associated factor 15 (*TAF15*), senataxin (*SETX*), and RNA-binding protein EWS (*EWSR1*), have also been linked to impaired DNA damage repair, further implicating the breakdown of this process in ALS pathogenesis [74–76].

2.4. Axonal Transport Defects

Axonal transport defects are a common observation in various neurodegenerative diseases, and mutations in components of the axonal transport machinery have unequivocally shown that impaired axonal transport can cause neurodegeneration [77]. The underlying cause of axonal transport defects in ALS is not fully understood [78]. Several mechanisms by which axonal transport may be perturbed in sporadic ALS and familial ALS by mutations in non-axonal transport genes have been proposed based mainly on studies of mutant *SOD1*-related ALS [79]. These include reductions in [1] microtubule stability, [2] mitochondrial damage, [3] pathogenic signaling (which alters phosphorylation of molecular motors and thereby regulate their function or through phosphorylation of cargo, such as neurofilaments, to disrupt their association with motors), and [4] protein aggregation [79,80]. There is evidence that TDP-43 may also be involved in the axonal transport defects seen in ALS [27]; axonal transport defects are commonly seen in neurodegenerative diseases [78]. TDP-43 has an important role in regulating axonal growth and impairment in posttranscriptional regulation of mRNAs in the cytoplasm of motor neurons [81]. Mutations in the genes coding for axonal transport first came to light when LaMonte et al. showed that disruption of the dynein/dynactin interaction by postnatal overexpression of p50/dynamitin, a 50-kDa subunit of dynactin encoded by *DCTN2*, caused reduced axonal transport in motor neurons and consequently led to a late-onset progressive motor neuron disease phenotype in the transgenic mice [82]. This study was followed by several studies showing that loss-of-function mutations in *DCTN1* cause a slowly progressive autosomal dominant distal hereditary motor neuropathy with vocal paresis (HMN7B) and ALS [79,83,84]. One key role for dynein in the neuron may be the removal of misfolded or degraded proteins from the cell periphery and the transport of these proteins back to the cell body for degradation. Dynein is also involved in the accumulation of misfolded proteins into aggresomes [78]. The next major family of microtubule-based molecular motors is kinesin. Kinesin moves mostly toward the plus end of microtubules, and was the first axonal transport motor to be identified. Now known as kinesin-1, it is a unidirectional motor, driving plus end-directed motility along microtubules in vitro [85]. Microtubules (e.g., α-tubulin) play a pivotal role in developing and maintaining neuronal cell structure and function, and they serve as essential tracks for both fast and slow long-distance axonal transport [86]. Several variants of the α-tubulin gene (i.e., *TUBA4A*), which destabilize the microtubule network and diminish its re-polymerization capability, have been identified as possible causes of ALS [87]. Whether these mutations affect axonal transport has not yet been determined, but since axonal transport prefers stable microtubules, they will likely have a detrimental effect [88].

2.5. Altered RNA Metabolism

As key regulators of RNA metabolism, RNA-binding proteins (RBP) play a critical role in maintaining the normal function of neuronal systems. RNA-binding proteins are involved in several aspects of RNA metabolism, including splicing, transcription, transport, translation, and storage in stress granules [89]. The aggregation of RBP is a pathological hallmark of amyotrophic lateral sclerosis and frontotemporal lobar degeneration. Interestingly, many of the ALS-linked RNA-binding proteins contain prion-like domains that are involved in stress granule formation or dynamics, including TDP-43, FUS, TAF15, ESWR1, hnRNPA1, and hnRNPA2B1 [90]. Mutations in ALS genes contribute to the etiology of FTD and vice versa [10]. Many ALS-causing mutations impact proteins involved in RNA metabolism, including RNA-binding proteins such as TDP-43, FUS, and heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) [91]. These and related RNA-binding proteins are components of organelles without membranes found in the nucleus (e.g., nuclear speckles and nucleoli) and cytoplasm (e.g., processing bodies and stress granules) in neurons and other cell types [92–94].

2.6. Mechanisms Leading to Dysregulation of RBP in ALS

Mutations in genes encoding many RBP are highly associated with ALS. In addition, dysregulation of RBP as a result of compromised nucleocytoplasmic trafficking, posttranslational modification (PTM), aggregation, and sequestration by abnormal RNAs also contribute significantly to disease pathogenesis. This section will briefly discuss the underlying mechanisms resulting in RBP dysregulation in ALS [95].

In response to a variety of stressors such as heat shock and oxidative insult, TDP-43 and FUS translocate from the nucleus and associate with cytoplasmic stress granules (SG), which are dense aggregations of protein-RNA complexes [96,97]. RBPs recruited to stress granules under conditions of chronic stress are capable of forming insoluble protein aggregates, even when other components of the stress granules have dissociated from the complex [98]. These granules facilitate cell survival by the translational arrest of non-essential transcripts and pro-apoptotic proteins when under stress [99]. Prion-like domains are thought to be vital for the reversible assembly of stress granules due to their capacity to form multiple transient weak interactions [90]. RBP also contains low complexity sequence domains (LCD), i.e., a glycine-rich domain that promotes protein aggregation [100] and contains RNA-recognition motifs (RRM) necessary for the nucleic acid binding functions of the protein [101]. Each protein also contains a nuclear localization sequence (NLS) that directs the subcellular localization of the protein to the nucleus under normal conditions [102]. Mutations in genes encoding NLS and LCD (Figure 1) lead to cytoplasmic retention and inclusion formation in cultured cells [103]. More than 250 proteins with aggregation-prone properties that are likely to contribute to neurodegeneration have been identified [104].

Low Complexity sequence Domains (LCD), Nuclear Localization Sequence (NLS). Mutations that occur in these domains (LCD and NLS) can trigger the same pathological cascade, which leads to a deterioration in the dynamics of stress granules (updated following original citation Baradaran-Heravi et al., 2019).

These studies suggest that such RBP could or should be considered as potential functional candidate genes in genetic studies. *RBM45*, an RNA-binding protein, is most likely a causal gene for ALS-FTD. In addition, a novel and evolutionary conserved structural element homo-oligomer assembly (HOA) domain has been identified. It is located within the linker region between RNA-recognition motifs (RRM2 and RRM3), which are essential for the self-association and oligomerization of *RBM45* (Figure 2). Since *RBM45* contains three RRM domains, it may associate with TDP-43 and FUS through RNA-protein interactions [105].

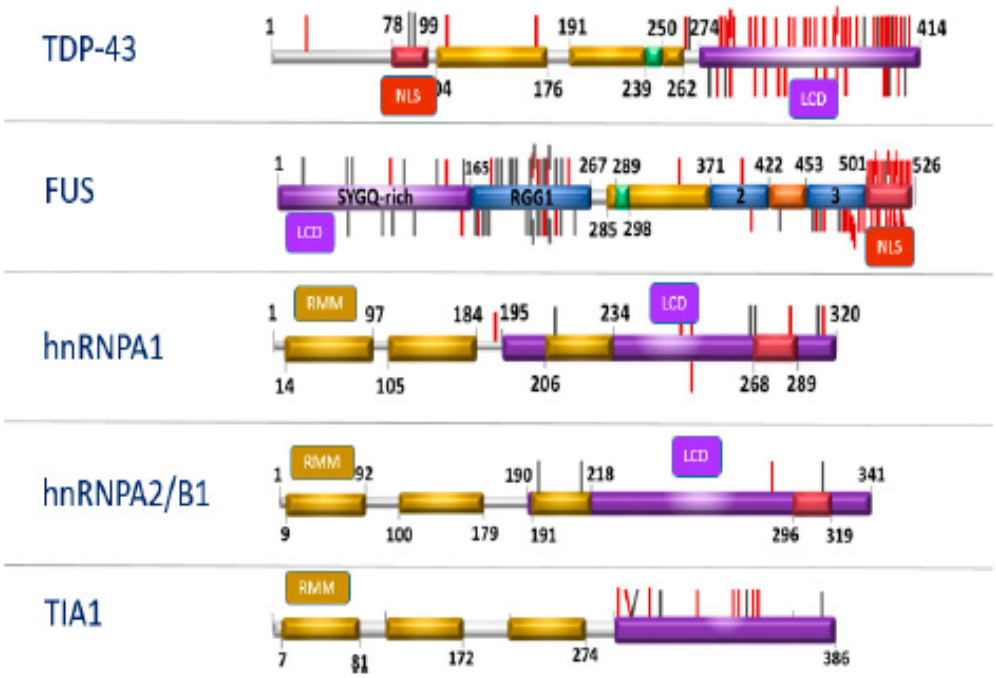

Figure 1. Structure of RNA-binding protein (RBP) genes.

Figure 2. Structure of the RBM45 gene.

RBM45 lacks the typical low complexity domain (LCD), which is actually common in RBPs; it has a suspicious homo oligomerization domain that, similar to LCD, mediates self-assembly through homo oligomerization and interaction with other proteins (updated following original citation Li et al. (2015).

Another RBP is *TIA-1* that promotes the assembly of stress granules discrete cytoplasmic inclusions into which stalled translation initiation complexes are dynamically recruited in cells subjected to environmental stress [106]. *TIA-1* is a modular protein composed of three RNA recognition motifs and a carboxy-terminal glutamine-rich motif that is structurally related to prion protein (PRD). Overexpressed *TIA-1* induces SG formation and represses reporter gene expression, whereas the isolated prion-related domain (PRD) of *TIA-1* forms cytoplasmic microaggregates [107]. These data suggest that the PRD is capable of self-oligomerization, just like *RBM45*.

2.7. Neuroinflammation

The inflammatory environment associated with ALS changes with disease progression and involves both neurotoxic and neuroprotective aspects. Neuroinflammation associated with neuronal loss is characterized by microglia and astrocyte activation, overproduction of inflammatory cytokines, and infiltration of T lymphocytes [108]. The secretion of inflammatory proteins by activated microglia leads to the potentially neurotoxic activation of astrocytes, which may contribute to the death of neurons and oligodendrocytes [109]. Genes that influence these functions are highly expressed in microglia and include *C9orf72*, *TBK1*, *OPTN*, *SQSTM1*, and *PGRN* [27,110–112]. Recent preclinical studies suggest that dys-

function of the gastrointestinal tract may also play a role in ALS pathogenesis by modifying the gut microbiota-brain axis [113]. Microglia and astrocytes in the central nervous system were shown to be regulated by metabolites derived from symbiotic gut microbes; the pathway inhibited neuroinflammation and neurodegeneration in an experimental autoimmune encephalomyelitis model [114,115]. Generation of low molecular weight metabolites by the gut microbiome is one postulated mechanism; these compounds are capable of passing through the blood-brain barrier and influencing neuronal function [116–118]. A correlation between ALS and altered composition of the gut microbiota was previously tested in animal models [117,119]. Several preliminary studies have analyzed the fecal microbiota in ALS patients, but with no conclusive results [120,121]. Whether the gut microbiome influences ALS is still controversial and remains a matter of debate.

3. Overview of ALS and FTD Genes

The advent of next-generation sequencing technologies, such as whole-genome sequencing (WGS) and whole-exome sequencing (WES), has led to a wave of novel genes associated with ALS [122,123]. A recent study found that multiple genetic variants can interact simultaneously to increase ALS susceptibility; these oligogenic cases of ALS may not appear familial in a conventional Mendelian sense; nonetheless, they may underlie the sporadic form of the disease [124–126]. More than forty-six ALS-related genes overlap with ALS genes linked to hereditary spastic paraplegia (HSP), FTD, mitochondrial disease, and lower motor neuropathies (LMN) [127] (Figure 3). Most of the heritability of FTD is accounted for by autosomal dominant mutations in three genes: Progranulin (*GRN*), microtubule-associated protein tau (*MAPT*), and *C9orf72* [128]. In recent years, an increasing number of mutations in other genes have been associated with autosomal dominant FTD, e.g., *VCP* (2004), *CHMP2B* (2005), *TARDBP* (2008), *FUS* (2009), *SQSTM1* (2012), *CHCHD10* (2014), *TBK1* (2015), *OPTN* (2015), *CCNF* (2016), and *TIA1* (2017). Recent studies have identified TBK1 as probably the fourth most common genetic cause overall of FTD, accounting for between 1% and 2% of all cases (although the pathogenic nature of many of the reported missense variants remains unclear) [129]. The first ALS gene, cytosolic superoxide dismutase (*SOD1*), was reported in 1993 [130] as well as other genes such as TAR DNA binding protein (*TARDBP*) [131–134], angiogenin (*ANG*) [135], fused in sarcoma (*FUS*) [35,36], optineurin (*OPTN*) [136], and the recently described chromosome 9 open reading frame 72 (*C9orf72*) [26,137]. An overview of recently proposed ALS genes that were identified based on rare genetic variants (*TBK1, CHCHD10, TUBA4A, CCNF, MATR3, NEK1, C21orf2, ANXA11, TIA1*) and their potential relevance to the genetic etiology of frontotemporal dementia have also been described [10].

3.1. SOD1

Mutations in *SOD1* account for the second most common cause of fALS after *C9orf72* [122]. *SOD1* mutations account for 15–20% of fALS pedigrees [138,139] and, until the discovery of *C9orf72*, was the most commonly identified gene in ALS. In most families harboring *SOD1* gene mutations, disease penetrance is >90% by age 70 yrs [140], and more than 170 mutations have now been detected in the fALS *SOD1* gene [141]. This likely remains true in many non-white populations, where *C9orf72* is much less common. Although multiple hypotheses have been proposed to explain mutant *SOD1*-mediated toxicity [142], the exact mechanism(s) responsible for motor neuron degeneration remains unresolved. Mitochondrial dysfunction is thought to contribute to the pathogenesis [143], and a proportion of the predominantly cytosolic *SOD1* has been reported to localize to mitochondria under certain conditions [144–146]. Pickles et al. reported that wild-type *SOD1* proteins are only partially located in the mitochondria, while mutant proteins show an increased propensity to be located in mitochondria, suggesting mitochondria involvement in the ALS etiology [147]. Another key player that directly interacts with *SOD1* is voltage-dependent anion channel 1 (VDAC1), a mitochondrial channel protein. Translocation of ions and proteins between mitochondria and the cytoplasm may be affected by mitochondria-associated misfolded

mutant *SOD1* [148], leading to increased mitochondria dynamic abnormalities and fragmentation [149]. Pure upper motor neuron (UMN) and lower motor neuron (LMN) forms have also been described, representing opposite clinical ends of the MND spectrum [9]. Mutations in the SOD1 gene could be associated with significant LMN involvement with or without signs of UMN. The A4V missense mutation occurs in around 40% of patients in North America, but is rare in the European population. LMN signs predominate, with features of UMN being mild or absent. Disease progression is particularly rapid, with a median survival of 1.2 years from disease onset [150]. The A4T mutation is also associated with a similarly rapid disease course for LMN predominant syndrome [151]. In contrast, the G93C mutation has been associated with a pure clinical phenotype of LMN, i.e., without bulbar involvement and a more favorable prognosis (i.e., a median survival of 153 months) [152].

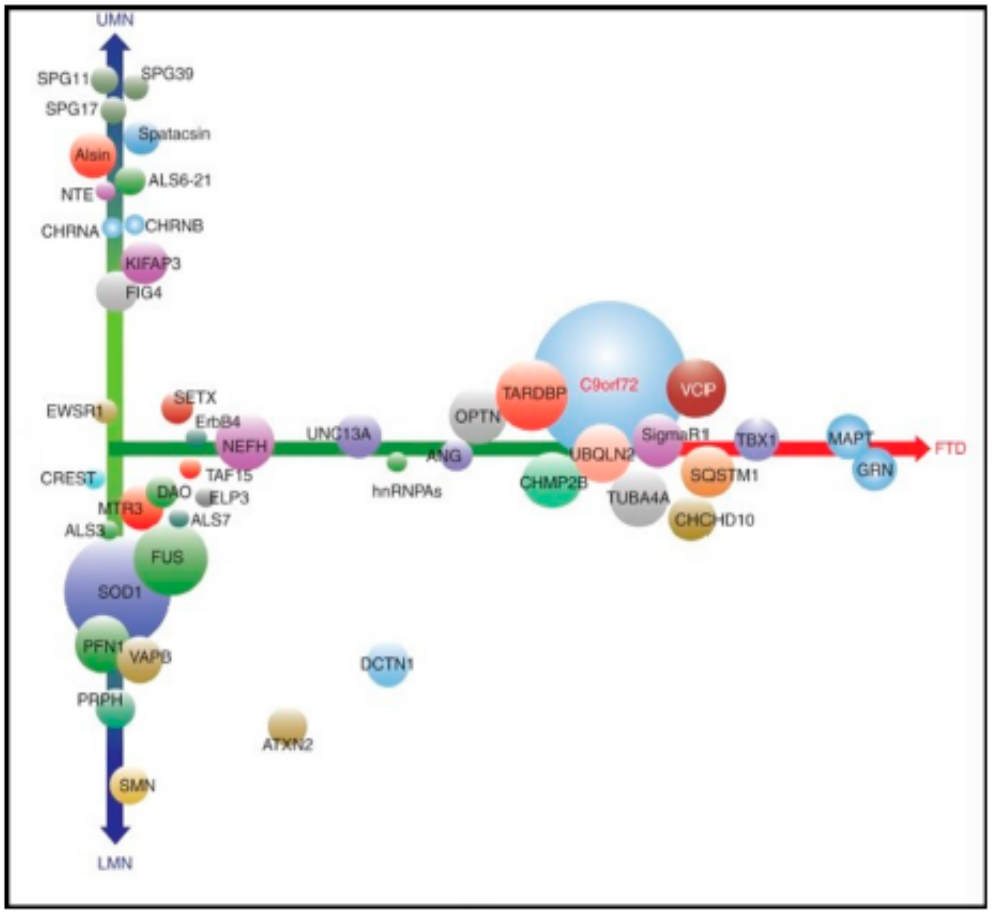

Figure 3. ALS-related genes. Symbols: LMN: Lower motor neuropathies, FTD: Frontotemporal dementia, UMN: Upper motor neurons. X-axis: Overlap with frontotemporal dementia, Y-axis: The extent to which corticospinal versus lower motor neurons are involved (updated following Ghasemi M. 2018).

3.2. *TARDBP and FUS*

One consistent pathologic finding in ALS and FTD is the presence of heavily ubiquitinated neuronal cytoplasmic inclusions, which in 2006 were found to contain TDP-4 [153]. TDP-43, an RNA-binding protein, is implicated in multiple aspects of RNA processing, including regulation of transcription, splicing, transport, and mRNA stabilization [154]. Significant modifications of TDP-43 have been identified as being hyperphosphorylated

and proteolytically cleaved by caspases. Activation and cleavage of TDP-43 is a key molecular step linking cellular redistribution and toxicity to the neurodegeneration observed in TDP-43 proteinopathies [155]. TDP-43 has a promiscuous protein interaction pattern with more than 200 targets reported, suggesting an involvement in a vast array of intracellular events [156]. Abnormal molecular weight TDP-43 fragments have been observed in neurons and astrocytes in patients with a spectrum of neurodegenerative diseases, including 95% of familial and sporadic ALS [157], making it an interesting candidate for all forms of the disease. *TARDBP* mutations were initially identified [131] as a direct consequence of the identification of the TDP-43-derived protein species as the principal constituent of the aggregates found in the upper and lower motor neurons of ALS patients without *SOD1* mutations and in FTLD-UPS [158,159]. Whereas 5% of familial ALS patients have the *TARDBP* mutation, mutations are rarely found in FTLD and FTD-MND [132,160].

3.3. *C9orf72*

The protein encoded by *C9orf72* is mainly related to autophagy, endosomal transport, and immune function. According to statistics, about 40–50% of fALS and 10% of sALS patients carry the *C9orf72* expanded alleles. In one study, the *C9orf72* expansion accounted for 11.7% of familial FTD [26]. The pathogenic alleles of *C9orf72* may have hundreds or even thousands of GGGGCC hexanucleotide repeats. A large number of clinical studies have shown that about 700–1600 GGGGCC hexanucleotide repeats are inserted into the intron located between the two untranslated optional exons 1a and 1b of the *C9orf72* gene [127,161–163]. Disease penetrance of *C9orf72*-related ALS is thought to be nearly 100% by the age of 80 yrs. No predictions for individual phenotypes, i.e., ALS, FTD, or ALS/FTD, the exact age at onset, the disease course, and disease duration is currently possible [162].

4. Novel ALS Genes

4.1. *KIF5A*

Kinesins are microtubule-based motor proteins involved in the intracellular transport of organelles within eukaryotic cells. *KIF5* genes are expressed in neurons, and transcription products function to transport cargo by binding to distinct adaptor proteins [164]. The central role of kinesins in axonal transport leads us to speculate that mutations in *KIF5A* cause disease by disrupting axonal transport. *KIF5* is responsible for the axonal transport of neurofilaments [165], and *KIF5A* knockout mice display abnormal neurofilament transport [166]. Abnormal accumulation of neurofilaments is a pathological hallmark of ALS, and rare mutations in the neurofilament heavy polypeptide (NEFH) are associated with ALS [167]. *KIF5* also contributes to the transport of mitochondria [164], and the impaired mitochondrial transport and function is another common hallmark of ALS patients [168–171].

4.2. *TBK1 and OPTN*

The *TBK1* and *OPTN* genes encode functionally related proteins that recently gained increased attention from the ALS research community. TBK1 (tumor necrosis factor (TNF) receptor-associated factor NF-κB activator (TANK)-binding kinase 1), also known as NAK or T2K, recently attracted the attention of human geneticists, immunologists, and neurologists alike for its critical role in pathologies of the central nervous system (CNS). *TBK1* is involved in the activation of various cellular pathways leading to IFN and pro-inflammatory cytokine production following infection [172], autophagic degradation of protein aggregates or pathogens [173,174], and homeostatic cellular functions such as cell growth and proliferation [175]. The majority of *TBK1* mutations are loss-of-function (LOF), leading to loss of the mutant transcript through nonsense-mediated mRNA decay (NMD) [10]. *TBK1* LOF mutations account for 3–4% of ALS-FTD patients [10]. Nonsense and frameshift mutations cause major disruptions to *TBK1* and may decrease its expression at both the mRNA and protein level, implying that *TBK1* haploinsufficiency contributes to the development of ALS [111,176].

4.3. CHCHD10

Many additional Coiled-coil-helix-coiled-coil-helix domain-containing protein 10 (*CHCHD10*) variants are now known to cause ALS, FTD, and other related degenerative diseases [177,178]. However, the degree of their pathogenicity and penetrance is undetermined. Variants causing ALS have been described; however, experimental evidence does not support the assumption that all disease-causing variants have the same mode of action [179]. *CHCHD10* G58R and *CHCHD10* G66V were identified in mitochondrial myopathy and late-onset spinal muscular atrophy [180–182] such as *VCP* and Matrin 3 (*MATR3*), which also exhibit clinical pleiotropy, including myopathy [183,184]. *CHCHD10* expression in patient tissues is unaffected, and *CHCHD10* S59L overexpression causes mitochondrial defects similar to those in affected patients [68]. This suggests that *CHCHD10* S59L is a dominant gain-of-function mutation [185]. To date, no published study supports routine diagnostic or predictive testing for *CHCHD10* variants in pure ALS [178].

4.4. MATR3

Matrin 3 is a highly conserved, inner nuclear matrix protein with two zinc finger domains and two RNA recognition motifs (RRM). It has been proposed that it stabilizes certain messenger RNA species [186]. Another study suggests that *MATR3* regulates alternative splicing events by binding to introns flanking repressed exons [10,187]. *MATR3* mutations are observed in 0.5–2% of ALS patients [188–190], but no studies so far have identified any *MATR3* mutations in FTLD [10].

4.5. HNRNPA1 and HNRNPA2B1

Over the last decade, dysfunction of hnRNPs has become closely linked to neurodegenerative diseases, most prominently amyotrophic lateral sclerosis and frontotemporal dementia, two diseases with significant genetic and pathological overlap [91]. hnRNP A1 and hnRNP A2B1 share similar domain architectures and are primarily localized in the nucleus [191–193]. hnRNP A2B1 are components of RNA transport granules found in neurons [194]. In addition, hnRNP A1 and hnRNP A2B1 translocate to the cytoplasm in response to stress and are recruited to stress granules [195,196]. hnRNP A1 and hnRNP A2B1 are associated with <1% of familial and sporadic forms of ALS; instead, they are more frequently associated with the broader spectrum multisystem proteinopathy (MSP) disorder [192,197].

4.6. TIA1

As a gene that has only recently been associated with ALS, *TIA1* harbors several ALS- and ALS/FTD-associated mutations in the low-complexity sequence domain (LCD) [31]. Notably, the LCD of *TIA1* is also the site of a mutation that causes Welander distal myopathy, a myopathy characterized by TDP-43-positive inclusions [198] and p62 [199,200]. TIA1 assembles in organelles without membranes, e.g., stress granules [31].

4.7. NEK1 and C21orf2

NEK1 encodes a member of the highly conserved NIMA (never in mitosis gene A) kinase family. It is a serine/threonine kinase involved in cell-cycle regulation, ciliogenesis, mitochondrial membrane permeability, and DNA damage repair [201–203]. Interestingly, in DNA damage repair, *NEK1* was shown to interact with *C21orf2*, which was recently associated with an increased ALS risk [204–206] (Table 1). Mutations in both *NEK1* and *C21orf2* are linked to skeletal disorders and axial spondylometaphyseal dysplasia [206]. Additionally, *C21orf2* may participate in the DNA repair process via interaction with *NEK1* [204]. Loss of function (LOF) variants account for about 1% of patients, and an interpretation of the pathogenicity and penetrance is complicated by the observation of occasional LOF variants in asymptomatic carriers [206]. In neurons, NEK proteins take part in maintaining the cytoskeleton network [207–209], which was previously linked to an ALS etiology via *TUBA4A* and *PFN1* [87,208,210]. With only 5–10% of sporadic ALS

cases harboring disease-associated mutations in known ALS genes [211], the remainder of sALS cases are presumed to represent a complex disease process influenced by both genetic and environmental exposures. Efforts to identify genetic risk factors have largely focused on genome-wide, and candidate gene association studies have met with only occasional success. Variants of the *UNC13A* gene, for example, have been associated with susceptibility to ALS and shorter survival times [212]. Intermediate length trinucleotide repeat expansions of both the *ATXN1* and *ATXN2* genes also increase the risk of disease, particularly for *C9orf72* expansion carriers in the case of *ATXN1* [213,214]. A copy number variation in the *EPHA3* gene, in contrast, has been flagged as a potential protective factor for ALS [215].

Table 1. Overview of recent ALS genes in different form ALS and TDP.

Gene	Locus	Inheritance	Mutation Freguency						Protein Function
			fALS %	sALS %	Overal ALS %	fTDP %	sTDP %	Overal TDP %	
TBK1	12q14.2	AD	3	<1	1.3	2	1	<1	autophagy
CHCHD10	22q11.23	AD	2	<1	<1	<1	<1	<1	mitochondrial dysfunction, synaptic integrity
TIA1	2p13.3	AD	2		<1				RNA metabolism
ANXA11	10q22.3	AD	1.2	<1	1.1				apoptosis, exocytosis, cytokinesis
CCNF	16p13.3	AD	0.6–3.3	<1	<1			4	UPS
NEK1	4q33	?	1–2	<1					DNA damage, mitochondrial membrane regulation
C21orf2	21q22.3	?	<1	<1	<1				DNA damage
MATR3	5q31.2	AD	1	1	<1				RNA metabolism
TUBA4A	2q35	AD	1	<1	<1	<1		<1	cytoskeletal dynamics

AD, autosomal dominant; UPS, ubiquitin-proteasome system.

5. Oligogenic/Polygenic Sporadic ALS

Variants in these genes are more likely to have a significant effect, and having the genotype greatly increases the probability of ALS; in other words, these variants show moderate to high penetrance; however, gene variants with low penetrance are also of interest, even though they only modestly increase the risk for any individual [216]. In at least some cases, ALS can be oligogenic, with affected individuals carrying more than one rare variant implicated in ALS [126,217]. An oligogenic basis of amyotrophic lateral sclerosis is debatable, since the presence of some variants have an uncertain significance [218]. However, a combination of a known pathogenic variant with one of uncertain significance is considered oligogenic inheritance by some [219]. What we do know is that oligogenic/polygenic sporadic ALS cases showed an earlier age of onset [220].

6. Future Questions

Our understanding of the biological and genetic basis of ALS, as well as our ability to care for ALS patients, has improved substantially over the last few years. Genetic causes of ALS have been identified in both sporadic and familial patients, and the number of disease-associated genes is still increasing [162]. One might assume that most of the monogenic forms have already been identified, but the issue of "heritability" is still unresolved, which might be explained by rare variants with large effect sizes [221]. The identification of genetic causes of ALS will help develop new therapeutic approaches, either through the identification of shared disease pathways such as the TDP-43 pathology or by targeted therapies for known mutations [222]. Currently, antisense oligonucleotide trials in SOD1- and *C9orf72*-related ALS are being conducted [223]. Besides riluzole, a second medication, edaravone, was approved by the FDA last year [224]. Whether it is beneficial to all

ALS patients or just to subgroups needs to be evaluated over the next few years [2]. In the future, biomarkers will hopefully help monitor disease progression and genomics, and transcriptomics will help to further personalize treatment based on each patient's individual disease subtype. For now, we need to define the molecular mechanisms that link specific disease-causing mutations to stress granule dysfunction and the accumulation of pathological inclusions.

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13.4. ANALÝZA A MAPOVÁNÍ VARIANT V GENU TIA1 ASOCIOVANÝCH S ALS/FTD

Yalda Baradaran-Heravi , Lubina Dillen , Hung Phuoc Nguyen, Sara Van Mossevelde , Jonathan Baets , Peter De Jonghe , Sebastiaan Engelborghs , Peter P De Deyn , Mathieu Vandenbulcke , Rik Vandenberghe , Philip Van Damme , Patrick Cras Eric Salmon , Matthis Synofzik , Peter Heutink , Carlo Wilke , Javier Simon-Sanchez , Ricard Rojas-Garcia , Janina Turon-Sans , Alberto Lleó , Ignacio Illán-Gala , Jordi Clarimón , Barbara Borroni , Alessandro Padovani , Pau Pastor , Monica Diez-Fairen , Miquel Aguilar, Ellen Gelpi , Raquel Sanchez-Valle , Sergi Borrego-Ecija , Radoslav Matej , Eva Parobkova , Benedetta Nacmias, Sandro Sorbi , Silvia Bagnoli , Alexandre de Mendonça [21], Catarina Ferreira , Matthew J Fraidakis , Janine Diehl-Schmid , Panagiotis Alexopoulos [23], Maria Rosário Almeida , Isabel Santana , Christine Van Broeckhoven , Julie van der Zee , BELNEU Consortium; EU EOD Consortium. No supportive evidence for TIA1 gene mutations in a European cohort of ALS-FTD spectrum patients.

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Negative results

No supportive evidence for *TIA1* gene mutations in a European cohort of ALS-FTD spectrum patients

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ABSTRACT

We evaluated the genetic contribution of the T cell–restricted intracellular antigen-1 gene (*TIA1*) in a European cohort of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) patients. Exonic resequencing of *TIA1* in 1120 patients (693 FTD, 341 ALS, 86 FTD-ALS) and 1039 controls identified in total 5 rare heterozygous missense variants, affecting the *TIA1* low-complexity domain (LCD). Only 1 missense variant, p.Met290Thr, identified in a familial FTD patient with disease onset at 64 years, was absent from controls yet received a combined annotation-dependent depletion score of 11.42. By contrast, 3 of the 4 variants also detected in unaffected controls, p.Val294Glu, p.Gln318Arg, and p.Ala381Thr, had combined annotation-dependent depletion scores greater than 20. Our findings in a large European patient-control series indicate that variants in *TIA1* are not a common cause of ALS and FTD. The observation of recurring *TIA1* missense variants in unaffected individuals lead us to conclude that the exact genetic contribution of *TIA1* to ALS and FTD pathogenesis remains to be further elucidated.

1. Introduction

Exome sequencing in an unresolved European amyotrophic lateral sclerosis (ALS)–frontotemporal dementia (FTD) family with transactive response DNA-binding protein-43 (TDP-43) brain pathology identified a co-segregation missense mutation in the low-complexity domain (LCD) of the T cell–restricted intracellular antigen-1 gene (*TIA1*), affecting stress granule dynamics (Mackenzie et al., 2017). Subsequent genetic screening revealed 6 additional rare missense mutations affecting the LCD in 3 unrelated ALS and 2 ALS-FTD patients, all absent from controls, giving an overall mutation frequency of ~2% familial ALS and <0.5% sporadic ALS (Mackenzie et al., 2017). Aiming to replicate the reported genetic association of *TIA1* with ALS and ALS-FTD, we set up a genetic screen of *TIA1* in a European case-control series of ALS-FTD spectrum patients, also including pure FTD patients.

2. Materials and methods

We sequenced the entire *TIA1* coding region (exons 1–13) in a total of 1120 patients and 1039 controls originating from different European countries (detailed methodology description and ethical assurance provided in Supplementary Material). The patient cohorts were recruited in the framework of the Belgian Neurology (BELNEU) Consortium, the European Early-Onset Dementia (EU EOD) Consortium, and the Tübingen FTD Exome series, as described (Blauwendraat et al., 2017; van der Zee et al., 2013; van der Zee et al., 2017). DNA and medical/demographic information was included on all patients, comprising 693 patients diagnosed with FTD, 341 patients with ALS, and 86 patients with concomitant FTD and ALS (FTD-ALS).

3. Results

We identified a total of 5 rare (minor allele frequency [MAF] <1%) heterozygous missense variants, with only 1 variant (p.Met290Thr) absent from the control cohort (Fig. 1, and Supplementary Material Table S1). All 5 missense variants mapped to the conserved *TIA1* LCD region (exons 11–13).

The p.Met290Thr (rs116707801) was present in an Italian familial FTD patient (age at onset 64 years, age at death 67 years). In ExAC NFE, this variant is counted 3 times in 66,732 alleles and has a combined annotation-dependent depletion (CADD) score of 11.42. The second missense variant identified in patients, p.Val294Glu (rs769199100), was present in an isolated Italian ALS patient (onset age 39 years). It was reported only once in 66,732 alleles of the ExAC NFE data set and received a CADD score of 20.9. However, we also observed the p.Val294Glu variant in one of our 1039 control individuals, also from Italian origin (age at inclusion 58 years). Furthermore, we detected a heterozygous missense variant p.Ala381Thr (rs768554955) in another Italian control individual (age at inclusion 70 years). The p.Ala381Thr variant was observed once in 66,740 alleles in ExAC NFE (MAF 0.001%) with a CADD score of 22.4.

In addition to these rare coding variants, we detected 2 recurring missense variants with ExAC NFE MAFs just below 1%. The p.Gln318Arg (rs115611153) variant was present in 15 patients and 29 controls. Despite its recurrent presence in unaffected individuals, it was scored among the 1% most deleterious variants in the genome, with a CADD score of 23.5. The p.Asn357Ser (rs116621885) variant was present in 14 patient and 18 controls, and had a CADD score of 14.25.

No further low-frequency or common variants were detected.

4. Discussion

We evaluated the genetic contribution of *TIA1* in 693 FTD, 341 ALS, and 86 ALS-FTD patients, including 80 patients with

[1] Belgian Neurology (BELNEU) Consortium: The following neurologist of the BELNEU Consortium have contributed to the clinical and pathological phenotyping and follow-up of the Belgian patient cohorts: Johan Goeman, Dirk Nuytten (Hospital Network Antwerp, Antwerp); Anne Sieben, Jan L. De Bleecker, Patrick Santens (University Hospital Ghent, Ghent); Jan Versijpt, Alex Michotte (University Hospital Brussels, Brussels); Adrian Ivanoiu (Saint-Luc University Hospital, Brussels); Olivier Deryck, Bruno Bergmans (General Hospital Sint-Jan Brugge, Bruges); Christiana Willems, Nina De Klippel (General Hospital Jessa, Hasselt); Dirk Peeters (General Hospital Groeninge Kortrijk).

[2] European Early-Onset Dementia (EU EOD) Consortium: The following members of the EU EOD Consortium have contributed to the clinical and pathological phenotyping and follow-up of the patients at their site that were included in the EU EOD Cohort: Silvana Archetti (Biotechnology Laboratory, Department of Diagnostics, Brescia Hospital, Italy); Elisa Bonomi (Centre for Neurodegenerative Disorders, Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, Italy); Irene Piaceri (Department of Neuroscience, Psychology, Drug Research and Child Health - University of Florence, Florence, Italy); Camilla Ferrari (IRCCS Don Gnocchi, Florence, Italy); Frederico Simões do Couto, Ana Verdelho, Gabriel Miltenberger-Miltényi (Faculty of Medicine, University of Lisbon, Lisbon, Portugal).

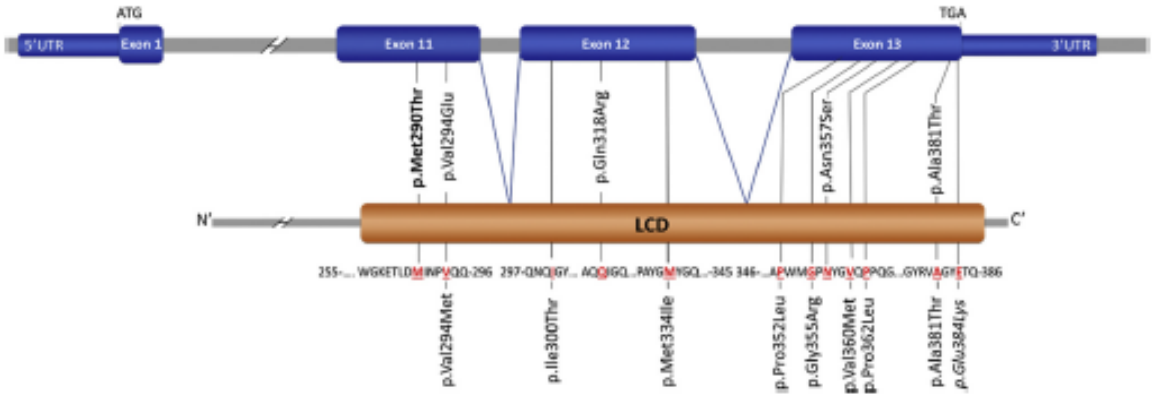

Fig. 1. Schematic representation of *TIA1* gene structure and protein with identified coding variants. Illustrated are the genomic region of *TIA1* exon 11-13 encoding for the low complexity domain (LCD). Variants identified in the present study are listed on top of the LCD structure; bold: patient-specific variant detected in a FTD patient, others: variants also detected in control individuals. Below the LCD structure, amino acid residues encoded by the respective exons and previously reported variants in ALS or ALS-FTD patients (Mackenzie et al., 2017; Yuan et al., 2017), as well as the Glu384Lys mutation identified in Welander distal myopathy (in italics) (Klar et al., 2013).

pathology-confirmed TDP-43 pathology. In line with Mackenzie et al., we did not identify any variants outside the LCD region. Only 1 variant was present in patients only, the other 4 were also observed in unaffected controls.

The patient-specific variant p.Met290Thr was observed in 1 male familial FTD patient of 693 FTDs (0.14%) and absent from a well-characterized control cohort of 1039 individuals. The CADD score however was significantly less than 20, indicating it is less likely to have a deleterious effect on protein function and explain the disease phenotype of the patient. In the absence of supportive co-segregation and functional evidence, we propose to classify this variant as variant of uncertain significance. We identified a female sporadic ALS patient with early disease onset of 34 years with a p.Val294Glu variant classified as deleterious by a CADD score of 20.9. However, we also detected the same variant in 1 male control individual of 58 years old. Of interest is that a different amino acid change at the same codon position (p.Val294Met, CADD score 22.3) was reported in an ALS patient by Mackenzie et al. and proposed it to be pathogenic (Mackenzie et al., 2017). We detected an additional missense variant, suggested to be pathogenic by Mackenzie et al. (p.Ala381Thr, CADD score 22.4), in one of our investigated controls with inclusion age of 70 years. In total, 3 of the 4 missense variants identified in unaffected control subjects had a CADD score >20, including the p.Gln318Arg variant present in 29 of our tested controls (CADD score 23.5).

Despite ours and others' ambiguous genetic observations (Van Der Spek et al., 2017), we acknowledge that *TIA1* is a promising functional candidate gene for TDP-related ALS and FTD.

Similar to other ALS and ALS-FTD genes, it encodes an RNA-binding protein that assembles into stress granules. Mutant *TIA1* was shown to alter these stress granule dynamics and, by this, promote TDP-43 accumulation and aggregation (Mackenzie et al., 2017).

Our findings in a large European patient-control cohort indicate that variants in *TIA1* are not a common cause of ALS and FTD. Furthermore, the observation of recurring *TIA1* LCD missense variants in unaffected individuals, including variants with estimated CADD scores >20, together with the lack of significant co-segregation in informative families, lead us to conclude that it is too early to attribute *TIA1* genetic variation to ALS or FTD risk.

Disclosure statement

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2018.05.005.

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Supplementary introduction

Amyotrophic lateral sclerosis (ALS) and Frontotemporal dementia (FTD) are fatal neurodegenerative disorders of the central nervous system (CNS). While ALS is defined by loss of upper and lower motor neurons leading to paralysis, FTD is characterized by atrophy of the frontal and temporal brain lobes giving rise to behavioral, cognitive and/or language dysfunction (Cruts et al., 2013). However, FTD patients can develop signs of motor neuron disease or ALS at some stage in the disease course. Equally, it is estimated that up to 50% of ALS patients show signs of behavioral dysfunction and/or subtle cognitive impairment resembling dementia, and up to 15% of ALS patients reach the diagnostic criteria of FTD referred to as FTD-ALS or ALS-FTD (Burrell et al., 2011; Ringholz et al., 2005; Wheaton et al., 2007). Both diseases are characterized by a high degree of genetic heterogeneity, with a significant number of overlapping genes. In ALS, over 30 genes have been identified, with the major ones - chromosome 9 open reading frame 72 (*C9orf72*), superoxide dismutase 1 (*SOD1*), TANK-binding kinase 1 (*TBK1*); FUS RNA binding protein *(FUS),* TAR DNA-binding protein gene (*TARDBP),* and valosin containing protein (*VCP*) - explaining 60-70% of patients with a positive family history of ALS (Renton et al., 2014). Likewise over ten genes have been associated with FTD, with the major contributors - *C9orf72,* granulin (*GRN*)*,* microtubule-associated protein tau (*MAPT*), *VCP* and *TBK1* - affecting about 40% of familial FTD patients (Blauwendraat et al., 2017; Gijselinck et al., 2012; Gijselinck et al., 2015; Sieben et al., 2012). Adding to this clinical and genetic overlap, aggregation of TAR DNA-binding protein 43 (TDP-43) in affected neurons and glial cells is the pathological hallmark of the majority of ALS (up to 97%) and FTD (up to 50%) patients (Ferrari et al., 2011; Ling et al., 2013; Mackenzie and Neumann, 2016).

A recent exome sequencing study revealed the *T cell-restricted intracellular antigen-1 gene* (*TIA1*) gene as a novel candidate gene for ALS and ALS-FTD (Mackenzie et al., 2017). In a European ALS-FTD family with TDP-43 neuropathology, a cosegregating *TIA1* p.Pro362Leu missense mutation was identified affecting a highly conserved amino acid residue in the low-complexity domain (LCD) of TIA1. Mutations in this domain had previously been associated with Welander distal myopathy (WDM), also characterized by aggregates of TDP-43 and p62 (Brand et al., 2016; Hackman et al., 2013; Klar et al., 2013). Subsequent genetic screening in unrelated patients revealed six additional rare missense mutations affecting the LCD in three ALS and two ALS-FTD patients, all absent from controls. Overall, *TIA1* mutations accounted for ~2% fALS and <0.5% sALS (Mackenzie et al., 2017).

Aiming to replicate the reported genetic association of *TIA1* with ALS and ALS-FTD, we set up a genetic screen of *TIA1* in a European case-control series of ALS-FTD spectrum patients, including also pure FTD patients.

Supplementary materials and methods

Study population

We sequenced the entire *TIA1* coding region (exon 1-13) in a total of 1120 patients and 1039 controls originating from different European countries. The patient cohorts were recruited in the framework of the Belgian Neurology (BELNEU) Consortium, the European Early-Onset Dementia (EU EOD) Consortium, and the Tübingen FTD Exome series, as described (Blauwendraat et al., 2017; van der Zee et al., 2013; van der Zee et al., 2017). DNA and medical/demographic information was included on all patients, comprising 693 patients diagnosed with FTD, 341 patients with ALS and 86 patients with concomitant FTD and ALS (FTD-ALS). Patients originated from Belgium (n=364), Spain (n=240), Italy (n=125), Germany (n=285), Portugal (n=47), Czech Republic (n=32), and Greece (n=27). Clinical diagnosis of

FTD, ALS and FTD-ALS/ALS-FTD was based on established international diagnostic criteria (Brooks et al., 2000; de Carvalho et al., 2011; Neary et al., 1998; Rascovsky et al., 2011; Schrooten et al., 2011). A positive family history was defined by at least one first- or second-degree relative with dementia or motor neuron disease and was observed in 33.19% (230/693) of the FTD group, 8.80% (30/341) of the ALS group and 30.23% (26/86) of the FTD-ALS group. Some patients received a pathology-confirmed diagnosis of TDP-43 positive pathology (Mackenzie and Neumann, 2016), which was the case for 5.77 % (40/693) of the FTD group, 6.45 % (22/341) of the ALS group and 20.93 % (18/86) of the FTD-ALS group. Overall, confirmed TDP-43 pathology was available for 7.14% (80/1120) of the patient population. From the BELNEU and EU EOD study populations, a control series was selected of 1039 individuals (69.5 +/- 9.9 years) without personal or family history of neurodegenerative disease, originating from Belgium (n=557), Spain (n=236) and Italy (n=246).

Ethical assurances

All research participants or their legal representatives signed informed consent for participation in clinical and genetic research. Clinical study protocols and informed consent forms were approved by the local medical ethics committees at the collaborating sampling sites. Genetic study protocols and informed consent forms were approved by the ethics committees of the University Hospital of Antwerp and the University of Antwerp, Belgium.

***TIA1* exonic resequencing**

For the full exonic resequencing of the *TIA1* gene (NM_022173), a multiamplicon target panel for amplification was designed using the amplicon target amplification assay (Agilent, https://www.agilent.com). Primers for multiplex PCR were designed using the mPCR primer design tool (or software) (Agilent, https://www.agilent.com) (Goossens et al., 2009). Specific target regions were amplified using multiplex PCR, followed by purification of the equimolar pooled amplicons using Agencourt AMPureXP beads (Beckman Coulter, CA, USA). Individual barcodes (Illumina Nextera XT) were incorporated in a universal PCR step before sample pooling. Bridge amplification and sequencing of barcoded samples was performed using an Illumina MiSeq platform, with the Illumina V2 reagent kit, generating 250bp paired-end reads. In addition, 174 whole exome sequencing (WES) datasets from FTD index patients from the Tübingen FTD Exome series was screened for *TIA1* coding variants. WES libraries were prepared using Agilent Technologies SureSelect V5 and subjected to 100 or 125-base pair paired-end sequencing on an Illumina HiSeq2000, HiSeq2500 or HiSeq4000 (for further methodological details (Blauwendraat et al., 2017).

The Burrows-Wheeler Aligner (BWA) tool was used to align and map reads against the human reference GRCh37 (hg19) (Li and Durbin, 2009). All 13 coding exons were successfully sequenced with 0% dropout and average coverage of ~1991x ranging from 133x to 4369x. Variant calling and annotation were carried out by Genome analysis Tool Kit (GATK) version 2.2, McKenna et al., 2010) in combination with GenomeComb (http://genomecomb.sourceforge.net/) (McKenna et al., 2010; Reumers et al., 2012). Identified initiation codon, missense, nonsense, indel and splice variants were selected based on minor allele frequency (MAF) for rare variants (MAF <1%), low frequent variants (MAF 1-5%) and common variants (MAF>5%) as reported for the Non-Finish European dataset of the Exome Aggregation Consortium (ExAC NFE) (http://exac.broadinstitute.org/). Raw reads of rare variants were manually checked using the integrative genomics viewer (IGV; Broad Institute, Cambridge, MA, USA). Selected variants were subsequently validated by direct Sanger sequencing on genomic DNA. Combined Annotation Dependent Depletion (CADD) scores were generated for functional annotation (http://cadd.gs.washington.edu/score) (Kircher et al., 2014).

Supplementary table

Table S1. *TIA1* missense variants identified in the present study

Genomic position	cDNA	Exon	Protein	Protein domain	dbSNP147	ExAC NFE MAF (allele count)	CADD	FTD n = 693	FTD-ALS n=86	ALS n=341	Controls n=1039
Variants in patients only											
g.70442521	c.869T>C	11	p.Met290Thr	LCD	rs116707801	0.004% (3/66732)	11.42	1			
Variants also observed in control individuals											
g.70442509	c.881T>A	11	p.Val294Glu	LCD	rs769199100	0.001% (1/66728)	**20.9**			1	1
g.70441561	c.953A>G	12	p.Gln318Arg	LCD	rs115611153	0.901% (600/66564)	**23.5**	8	1	6	29
g.70439941	c.1070A>G	13	p.Asn357Ser	LCD	rs116621885	0.961% (641/66730)	14.25	10	2	2	18
g.70439870	c.1141G>A	13	p.Ala381Thr	LCD	rs768554955	0.001% (1/66740)	**22.4**				1

gDNA numbering according to the human reference sequence (Genome Reference Consortium Human Build 37/ human genome 19, GRCh37/hg19); cDNA numbering according to the cDNA reference sequence NM_022173; Protein numbering relative to the TIA1 isoform NP_ 071505.2. CADD score: Combined Annotation Dependent Depletion score (http://cadd.gs.washington.edu). CADD scores above 20 are indicated in bold and considered to be amongst the top 1% most deleterious variants in the human genome (Kircher et al., 2014); dbSNP147: the Single Nucleotide Polymorphism Database version 147; MAF: Minor allele frequency; ExAC NFE: Non-Finish European dataset of the Exome Aggregation Consortium (http://exac.broadinstitute.org/).

Disclosure statement

None of the authors had a conflict of interest in respect with the data presented in this paper.

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14. SHRNUTÍ A ZHODNOCENÍ CÍLŮ PRÁCE.

Mutační screening genů u pacientů s sCJD a sCJD s další proteinopatií - diagnostika sporadické Creutzfeldtovy-Jakobovy nemoci a dalších proteinoaptií.

Studie tvořila skupinu 30 pacientů (n=11) s diagnózou „čistou „sCJD a sCJD a PART nebo raná fáze AD (úroveň kritérií konsensu NIA „nízká“) a sCJD s pokročilejší AN (konsenzus NIA) úroveň kritérií A2 a vyšší). V druhé části jsme metodou masivního paralelního sekvenování nové generace (NGS) analyzovali 15 genů, které se mohou podílet na rozvoji onemocnění. Identifikovali jsme polymorfizmus p.E318G v genu *PSEN1*, který byl nalezen u tří pacientů (10,3%), z nichž jeden měl čistý sCJD a dva tvořili skupinu sCJD + AD. U ostatních genů asociovaných s AN jako je *APP* a *PSEN2* nebyly pozorovány žádné relevantní varianty. V genu *PRNP* byla inzerce p.P84_Q91Q detekována u jednoho pacienta s p-amyloidopatií, nicméně tato varianta je považována za nepatogenní. Polymorfní variantu alelu e4 v genu *APOE* neslo 10% pacientů (n = 3). Všechny tři případy byly na úrovni AN A2 (AAO 62, 75, 83 let). Distribuce polymorfního kodonu 129 *PRNP* a *APOE* genotypu u pacientů s sCJD nevykazovala jednoznačnou asociaci mezi stavem alely APOE ε4 a sCJD; avšak APOE e4 byl pozorován u dvou homozygotů PRNP M129M (n = 2).

Naše studie nebyla zaměřena na problematiku sCJD a synukleinopatie, vzhledem k extrémně nízkému výskytu obou patologických stavů při komorbiditě. Tato otázka je nicméně slibným směrem pro budoucí výzkum a jako taková by nám mohla pomoci lépe porozumět genetickému pozadí a možná nabídnout nové terapeutické možnosti.

Naše pilotní studie jednoznačně neidentifikovala žádné kritické rozdíly mezi „čistou“ sCJD a sCJD ve spojení s dalšími komorbidními neurodegenerativními nemocemi.

Fenotypové a genotypové porovnání vzácných případů GSS zachycených v České republice.

Gestmannův-Sträusslerův-Scheinkerův syndrom je vzácné neurodegenerativní onemocnění způsobené mutací P102L v genu *PRNP*. Je velmi obtížné identifikovat pacienty postižené GSS, protože tato choroba je kvůli klinické podobnosti s jinými chorobami těžko diagnostikována. Doposud byla prevalence GSS odhadována na 1 až 10/100 000 000. Dle záchytu 7 případů (a následně dalšího případu již do studie nezahrnutého) v krátké časové periodě v ČR, lze ale teoreticky odhadovat prevalenci až na 8/10 000 000. Mnohé případy byly zachyceny náhodně pomocí genetického vyšetření (jejich symptomatika většinou neodpovídá „typickému GSS syndromu"). Studovali jsme pohlaví, věk při prvních projevech a dobu trvání onemocnění, nástup demence, trvání kognitivního deficitu, nástup ataxie, abnormality v bazálních gangliích, kortexu a mozečku na MRI/CT snímcích, polymorfizmus v kodonech 129 a 219, změny ve šlachově okosticových reflexech a v taktilním čití, a nález v mozkomíšním moku (základní likvorologický obraz, protein 14-3-3, tau, fosfo tau a bet amyloid). Na základě klinických, genetických a radiologických vyšetření byly klastrovou analýzou (metoda DBSCANu) odhaleny 4 subtypy GSS lišící se dobou nástupu nemoci, ataxie, demence, délkou trvání nemoci, senzitivními příznaky a reflexy. Výsledné fenotypy jsme označili následovně: 1) „typický GSS"; 2) „GSS s areflexií a paretéziemi"; 3) „GSS podobný CJD"; a 4) „GSS s demencí ". Námi nově definované klinické varianty GSS by mohly napomoci ve zlepšení diagnostiky doposud nedetekovaných případů tohoto vzácného onemocnění.

Komplexní analýza genů asociovaných s ALS/FTLD - podobnosti v genetických pozadích.

Existuje mnoho genů, které byly charakterizovány jako původce ALS, u kterých byly popsány potenciálně patogenní varianty i v případech FTLD. I když je to vzácné, společný výskyt ALS a FTD spojený s mutacemi v genech posiluje genetickou vazbu mezi těmito dvěma poruchami. Tyto geny kódují proteiny spojené se systémem autofagie / ubikvitin proteazomu (UPS) nebo RNA. Výjimkou je *TUBA4A*, který kóduje protein spojený s mikrotubuly. Kromě toho byly popsány i přechodné expanze CAG v *ATXN2* jako rizikový faktor v ALS a ALS-FTD a modifikátor nemoci v ALS i FTD. Použití sekvenování nové generace, ať už ve formě cíleného sekvenování celého exomu, nebo celého genomu (WGS), mělo významný dopad na identifikaci genů spojených s těmito chorobami. Zejména se rozšířilo spektrum nemocí, které mají souvislost s nalezenými variantami v těchto genech, dále také rozšíření spektra genů asociovaných s ALS a FTD a zvýšení frekvence variant známých genů ALS a FTD v rámci sporadických případů. Doufáme, že pracovní skupiny velkých mezinárodních skupin ALS a FTD, jako jsou Project MinE a GENFI jednou v budoucnu zodpoví, proč jednotlivci s konkrétní variantou pokračují ve vývoji ALS, FTD nebo ALS-FTD.

Analýza a mapování variant v genu TIA1 asociovaných s ALS/FTD

V rámci konsorcia EU Early Onset Dementia (EU EOD) jsme soubor 2159 pacientů s ALS-FTD sekvenovali pomocí cíleného vysoce výkonného sekvenačního panelu. Sekvenovali jsme celou oblast kódující TIA1 (exony 1-13). Identifikovali jsme celkem 5 vzácných (frekvence menších alel [MAF] <1%) heterozygotních missense variant. Provedli jsme mapování nalezených variant a zjistili jsme, že všechny varianty se vyskytují v konzervované doméně LCD (low complexity domain) a mohou ovlivnit dynamiku stresových granul. Rozšířili jsme objev mutací a analýzu vzácných variant na kohorty ALS / FTD z různých geografických populací. Naše genetické nálezy naznačují, že TIA1 hraje roli ve familiární i sporadické patogenezi ALS a i navzdory našich nejednoznačných genetických nálezů se domníváme, že TIA1 je slibný funkční kandidátský gen pro ALS a FTD.

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15. ABSTRACT

Genetics plays a crucial role in translational research, which ultimately aims to develop new therapies that modify neurodegenerative disorders. We anticipate that individual genetic profiling will become increasingly important in a clinical context with implications for patient care in line with the proposed ideal of personalized medicine. Although whole exome sequencing (WES) is now widely used in gene identification studies, there is no doubt that whole genome sequencing (WGS) will soon replace WES as the standard method for gene discovery. Hopefully, in the near future, comparable initiatives will be developed to generate global, fully genomic, publicly available data for various forms of neurodegenerative diseases such as FTD.

In the first part of this work we focused on a group of patients diagnosed with "pure" sCJD and sCJD and PART or early phase AD and sCJD with more advanced AD. We compared and analyzed a new generation sequencing (NGS) method of 15 genes that may be involved in the development of the disease. We identified a p.E318G polymorphism in the PSEN1 gene, which was found in three patients (10.3%), one of whom had pure sCJD and two were sCJD + AD. No relevant variants were observed for the other AD APP and PSEN2 genes. In PRNP, insertion of p.P84_Q91Q was detected in one patient with β-amyloidopathy, however, this variant is considered non-pathogenic. A polymorphic variant of the e4 allele in the APOE gene was carried by 10% of patients (n = 3). All three cases were at the AD A2 level (AAO 62, 75, 83 years). The distribution of polymorphic codon 129 of the PRNP and APOE genotypes in patients with sCJD did not show a clear association between the status of the APOE ε4 and sCJD alleles; however, APOE e4 was observed in two PRNP M129M homozygotes (n = 2). Our study did not focus on the issue of sCJD and synucleinopathy, due to the extremely low incidence of both pathological conditions in comorbidity. However, this question is a promising direction for future research and as such could help us better understand the genetic background and possibly offer new therapeutic options. Our pilot study clearly did not identify any critical differences between pure sCJD and other sCJD in association with other comorbid neurodegenerative diseases.

The aim of the secod part of the study was to a related neurodegenerative disease caused by the P102L mutation in the PRNP gene, called Gestmann-Sträussler-Scheinker. It is very difficult to identify patients with GSS because the disease is difficult to diagnose due to its clinical similarity to other diseases. So far, the prevalence of GSS has been estimated at 1 to 10 / 100,000,000. According to the detection of 7 cases in a short period of time in the Czech

Republic, the prevalence can be estimated at 8 / 10,000,000. In these cases, a family relationship was excluded. Many cases were detected randomly by genetic testing (their symptoms usually do not correspond to "Typical GSS syndrome"). We studied gender, age at first manifestations and duration of disease, onset of dementia, duration of cognitive deficit, onset of ataxia, abnormalities in basal ganglia, cortex and cerebellum on MRI / CT scans, polymorphism in codons 129 and 219, changes in tendon perimeter reflexes and in tactile sensation, and a finding in cerebrospinal fluid (basic cerebrospinal fluid, protein 14-3-3, tau, phosphotau and bet amyloid). Based on clinical, genetic and radiological examinations, 4 subtypes were observed by cluster analysis (DBSCAN method), differing in the time of onset of the disease, ataxia, dementia, duration of the disease, sensitive symptoms and reflexes. We designated the resulting phenotypes as follows: 1) "typical GSS"; 2) "GSS with areflexia and paretheses"; 3) "GSS similar to CJD"; and 4) "GSS with dementia". The newly defined clinical variants of GSS will hopefully help in the diagnosis of hitherto undetected cases.

In the third part of this work we characterized many genes whose mutations cause ALS. Potentially pathogenic variants of these genes have also been described in cases of FTD. Although rare, the co-occurrence of ALS and FTD associated with mutations in genes strengthens the genetic link between the two disorders. These genes encode proteins associated with the autophagy / ubiquitin proteasome (UPS) or RNA system. An exception is TUBA4A, which encodes a microtubule-associated protein. In addition, transient expansions of CAG in ATXN2 have been described as a risk factor in ALS and ALS-FTD and a disease modifier in both ALS and FTD. The use of next generation sequencing, whether in the form of targeted whole exoma or whole genome sequencing (WGS), has had a significant impact on the identification of genes associated with these diseases. In particular, the spectrum of diseases related to the variants found in these genes has expanded, as has the broadening of the spectrum of genes associated with ALS and FTD and the increase in the frequency of variants of known ALS and FTD genes in sporadic cases. We hope that the working groups of the large international groups ALS and FTD, such as Project MinE 2 and GENFI 3, will answer in the future why individuals with a specific variant continue to develop ALS, FTD or ALS-FTD.

In the last part of the study, we investigated within the EU Early Onset Dementia (EU EOD) consortium, we sequenced a set of 2159 patients with ALS-FTD using a targeted high-throughput sequencing panel. We sequenced the entire TIA1 coding region (exons 1-13). We identified a total of 5 rare (frequency of smaller alleles [MAF] <1%) heterozygous missense variants. We mapped the variants found and found that all variants occur in the conserved LCD

domain (low complexity domain) and can affect the dynamics of stress granules. We extended the discovery of mutations and the analysis of rare variants to ALS / FTD cohorts from different geographical populations. Our genetic findings suggest that TIA1 plays a role in the family and sporadic pathogenesis of ALS and, despite our ambiguous genetic findings, help that TIA1 is a promising functional candidate gene for ALS and FTD.

In conslusion, we would like to sum up that clinical course of genetic forms of neurodegenerative diseases can be completely different from sporadic types of diseases, and without knowledge of a pathogenic mutation, it is often not possible to make a correct diagnosis. The main problems in the search for new candidate genes are the different heterogeneity of phenotypes, genotypes and the potential gene - gene and gene - environment interactions, as described in many studies. Furthure studies shloud be performed in the near future to complete our understanding of all possible of gene interactions in neurodegenerations through network biology.

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17. SEZNAM POUŽITÝCH ZKRATEK

Použité zkratky genů

ANXA11	Annexin A11
AKAP9	A-kinase anchor protein 9
ATXN2	Ataxin 2
BPAN	Beta-propeller protein-associated neurodegeneration
C9orf72	chromosome 9 open reading frame 72
C21orf2	chromosome 21 open reading frame 2
CCNF	Cyclin F
DCTN1	Dynactin Subunit 1
FUS	Fused in Sarcoma
GRN	Granulin Precursor
CHCHD10	Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 10
KIF5A	Kinesin Family Member 5A
MAPT	Microtubule Associated Protein Tau
MATR3	Matrin 3
MPAN	mitochondrial membrane protein-associated neurodegeneration
NEK1	NIMA Related Kinase 1
OPTN	Optineurin
PFN1	Profilin 1
PKAN	Pantothenate Kinase-Associated Neurodegeneration
PLAN	PLA2G6-associated neurodegeneration
PRNP	Prion protein
ROS	Proto-oncogene tyrosine-protein kinase
SOD1	Superoxide dismutase
SQSTM1	Sequestosome 1
TARDBP	TAR DNA Binding Protein

TBK1	TANK-binding kinase 1
TIA1	T-cell intracellular antigen 1
TREM2	Triggering Receptor Expressed On Myeloid Cells 2
TUBA4A	Tubulin alpha-4A
UBQLN2	Ubiquilin-2
UNC 5C	unc-*5* netrin receptor C
VAPB	Vesicle-associated membrane protein-associated protein B/C
VCP	Valosin-containing protein

Použité zkratky

AGD	demence s argyrofilními zrny
ALS	amyotrofická laterální skleróza
AN	Alzheimerova nemoc
ASO	antisense oligonukleotidy
ARTAG	věkově vázaná astrogliopatie s depozity tau proteinu
BSE	bovinní spongiformní encefalopatií,
CAA	cerebrální amyloidová angiopatie
CBD	kortikobazální degenerace
CSF	mozkomíšní mok
GGT	globulární gliová tauopatie
CBS	kortikobazálnímu syndromu
CJD	Creutzfeldtova-Jakobova choroba
DM	dura mater
CTB	C-teminal domain
DLB	demence s Lewyho tělísky
DNA	deoxyribonukleová kyselina
fALS	familiární amyotrofická laterální skleróza
fCJD	familiární Creutzfeldtova-Jakobova choroba
FBD	familiární britská demence
FDD	familiární dánská demence
FFI	fatální familiární insomnie
FTD	frontotemporální demence
FTLD	frontotemporální lobární demence
FTLD-ALS	frontotemporální lobární demence a amyotrofické laterální sklerózy
FTLD-MND-TDP	frontotemporal lobar degeneration s onemocněním motorického neuronu a TDP-43 positivními inkluzemi
FTLD-tau	FTLD s tau pozitivními inkluzemi

fvFTLD	behaviorální varianta frontotemporální demence
GWAS	genome-wide association study
GCIs	cytoplasmatické inkluze oligodendogrií
GSS	Gerstmannův – Sträusslerův – Scheinkerův syndrom
HN	Huntingtonova nemoc
iCJD	iatrogenní Creutzfeldtova-Jakobova choroba
LATE	věkově vázaná limbická TDP-43 proteinopatie
LDL	nízkodenzitní lipoprotein
MSA	multisystémová atrofie
NBIA	neurodegeneration with brain iron accumulation
NCIs	inkluze podobné neurofibrilárním klubkům
NIA	National Institute of Aging
PART	primární na věk vázaná tauopatie
PPA	primární progresivní afázie
PrP	prionový protein
PSP	progresivní supranukleární obrna
ROS	Reactive Oxygen Species
sALS	sporadická amyotrofická laterální skleróza
sCJD	sporadická Creutzfeldtova-Jakobova choroba
SG	stresové granule
TA	astrogliálních cytoplasmatických inkluzí
TSE	lidské přenosné spongiformní encefalopatie
vCJD	variantní Creutzfeldtova-Jakobova choroba
VLDL	very low-density lipoprotein

18. PŘEHLED PUBLIKAČNÍ A ODBORNÉ AKTIVITY

Publikace k tématu

Amyotrophic Lateral Sclerosis and Frontotemporal Lobar Degenerations: Similarities in Genetic Background.

Parobkova E, Matej R. Diagnostics (Basel). 2021 Mar 13;11(3):509. doi: 10.3390/diagnostics11030509. PMID: 33805659; PMCID: PMC7998502.
IF 3.706

Sporadic Creutzfeldt-Jakob Disease and Other Proteinopathies in Comorbidity.

Parobkova E, van der Zee J, Dillen L, Van Broeckhoven C, Rusina R, Matej R. Front Neurol. 2020 Nov 30;11:596108. doi: 10.3389/fneur.2020.596108. PMID: 33329348; PMCID: PMC7735378. **IF 4,003**

Genetika Alzheimerovy nemoci a dalších neurodegenerativních demencí.

PAROBKOVÁ E., RUSINA R., MATĚJ R. In BRDIČKA R., HERLE P. (EDS.), Genetika: pro všeobecné praktické lékaře. Praha: Raabe, 2020, s. 180-190. ISBN 978-80-7496-447-3.

Clinical Variability in P102L Gerstmann-Sträussler-Scheinker Syndrome.

Tesar A, Matej R, Kukal J, Johanidesova S, Rektorova I, Vyhnalek M, Keller J, Eliasova I, **Parobkova E**, Smetakova M, Musova Z, Rusina R. Ann Neurol. 2019 Nov;86(5):643-652. doi: 10.1002/ana.25579. Epub 2019 Sep 4. PMID: 31397917. **IF 9,55**

Genetika neurodegenerativních demencí v deseti bodech – co může neurolog očekávat od molekulárního genetika?

E. Parobková, R. Rusina, M. Matějčková, V. Gregor, R. Matěj. Cesk Slov Neurol N 2019; 82(1): 100-105. doi: 10.14735/amcsnn2019100. **IF 0,3**

No supportive evidence for TIA1 gene mutations in a European cohort of ALS-FTD spectrum patients.

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Engelborghs S, De Deyn PP, Vandenbulcke M, Vandenberghe R, Van Damme P, Cras P, Salmon E, Synofzik M, Heutink P, Wilke C, Simon-Sanchez J, Rojas-Garcia R, Turon-Sans J, Lleó A, Illán-Gala I, Clarimón J, Borroni B, Padovani A, Pastor P, Diez-Fairen M, Aguilar M, Gelpi E, Sanchez-Valle R, Borrego-Ecija S, Matej R, **Parobkova E**, Nacmias B, Sorbi S, Bsoli S, de Mendonça A, Ferreira C, Fraidakis MJ, Diehl-Schmid J, Alexopoulos P, Almeida MR, Santana I, Van Broeckhoven C, van der Zee J; BELNEU Consortium; EU EOD Consortium. Neurobiol Aging. 2018 May 23. pii: S0197-4580(18)30161-1. doi: 10.1016/j.neurobiolaging.2018.05.005. Epub ahead of print]. PMID:29886022.

IF 4,3

Rare mutations in SQSTM1 modify susceptibility to frontotemporal lobar degeneration.

van der Zee J, Van Langenhove T, Kovacs GG, Dillen L, Deschamps W, Engelborghs S, Matěj R, Vandenbulcke M, Sieben A, Dermaut B, Smets K, Van Damme P, Merlin C, Laureys A, Van Den Broeck M, Mattheijssens M, Peeters K, Benussi L, Binetti G, Ghidoni R, Borroni B, Padovani A, Archetti S, Pastor P, Razquin C, Ortega-Cubero S, Hernández I, Boada M, Ruiz A, de Mendonça A, Miltenberger-Miltényi G, do Couto FS, Sorbi S, Nacmias B, Bagnoli S, Graff C, Chiang HH, Thonberg H, Perneczky R, Diehl-Schmid J, Alexopoulos P, Frisoni GB, Bonvicini C, Synofzik M, Maetzler W, vom Hagen JM, Schöls L, Haack TB, Strom TM, Prokisch H, Dols-Icardo O, Clarimón J, Lleó A, Santana I, Almeida MR, Santiago B, Heneka MT, Jessen F, Ramirez A, Sanchez-Valle R, Llado A, Gelpi E, Sarafov S, Tournev I, Jordanova A, **Parobkova E**, Fabrizi GM, Testi S, Salmon E, Ströbel T, Santens P, Robberecht W, De Jonghe P, Martin JJ, Cras P, Vandenberghe R, De Deyn PP, Cruts M, Sleegers K, Van Broeckhoven C.Acta Neuropathol. 2014 Sep;128(3):397-410. doi: 10.1007/s00401-014-1298-7. Epub 2014 Jun 5. PMID: 24899140

IF 18,17

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